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CARDITIS IN POLIOMYELITIS

AN ANATOMIC STUDY OF THIRTY-FIVE CASES AND REVIEW OF THE LITERATURE *

THEODORE E. LUDDEN, M.D., and JESSE E. EDWARDS, M.D.
(From the Mayo Clinic, Rochester, Minn.)

This investigation was undertaken because of the relative paucity of reports in the literature regarding cardiac lesions in cases of acute poliomyelitis and because we observed certain unusual cardiovascular lesions during the 1946 epidemic in Minnesota.

In 1910 Robertson and Chesley reported on the necropsies in 6 cases of acute poliomyelitis. In 5 the hearts were studied histologically and swelling of myocardial fibers and interstitial edema of the myocardium were noted. Even then these authors called attention to the lack of emphasis in the literature upon the changes in organs outside the nervous system.

Minimal myocardial inflammatory changes in cases of acute poliomyelitis were subsequently reported by Abramson, in 1918, and by Landon and Smith, in 1934. The latter authors stated that the observed lesions were similar to those occurring in diphtheria and scarlet fever, but apparently they did not feel that the changes were significant.

In 1934 Cowie, Parsons, and Lowenberg, reporting necropsy findings in 4 cases of acute poliomyelitis, included one case in which localized myocarditis of the right atrium, a mural thrombus at this site, and pulmonary emboli were observed.

Clark, in 1938, reported data on an interesting case of acute poliomyelitis in which severe diffuse interstitial myocarditis was found at necropsy. Since horse serum had been administered, with the subsequent development of typical signs of serum sensitivity, the myocarditis was attributed to horse serum.

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It remained for Saphir and Wile, in 1942, to point out that myocarditis is a feature of poliomyelitis. The histologic changes described by these authors in 6 of 7 cases included cellular exudate in the myocardium, consisting mainly of monocytes, adventitial cells, and lymphocytes, or of neutrophils and lymphocytes. Minimal degenerative changes also were described. Peale and Lucchesi, in 1943, reported the finding of essentially similar changes in 5 of 9 cases. In the same year Dublin and Larson reported the finding of 2 incidental cases of acute myocarditis among 12 cases of fatal acute poliomyelitis.

In 1945 Saphir published the results of a necropsy study on 17 additional cases of acute poliomyelitis, in 10 of which he found myocarditis. In 3 cases he noted histologic changes in the media of the aorta, consisting of separation of elastic lamellae by a substance which, he stated, had a fibrinoid appearance.

Luhan, in 1946, reported a series of 13 cases of fatal acute poliomyelitis, in one of which he found verrucous endocarditis involving the mitral valve. The heart was not further described.

Recently Gefter, Leaman, Lucchesi, Maher, and Dworin published a clinical and pathologic study of a group of 467 cases of acute poliomyelitis, in 6 of which necropsy was performed. Their investigation disclosed interesting facts concerning the incidence of cardiac murmurs, electrocardiographic changes, and other pertinent clinical findings related to the heart. Thirty-two (14.2 per cent) of 226 patients were found to have abnormal electrocardiograms. Myocarditis was found in 2 of the 6 cases in which necropsy was performed. Unfortunately, it had not been possible to take electrocardiographic tracings on these patients, because of the severity of their illnesses.

A survey of the literature on the pathology of poliomyelitis does not permit accurate determination of the incidence of significant cardiac changes, since the histopathologic findings in the heart are seldom described in the reports. After review of the few available studies in which the heart was specifically investigated, it would seem that cardiac lesions are common in cases of fatal acute poliomyelitis.

Material and Methods

The 35 cases used for the investigation herein reported represent all of the cases of poliomyelitis in which necropsy was performed at the Mayo Clinic, from August 2, 1925, to December 11, 1946, inclusive, and in which the hearts were available for this study. The specimens were sectioned for histologic investigation by the method used by Gross, Antopol, and Sacks in the study of rheumatic heart disease. Their method

was adopted primarily because it made possible, by simple standardized technics, the demonstration of most of the important anatomic structures of the heart. The hearts had all been fixed in 10 per cent formalin. Six blocks were cut from each specimen and embedded in paraffin. Sections were cut at 4 to 6 μ. Hematoxylin and eosin stains were used on sections from each block. Bodian's stain and Mallory's phosphotungstic acid hematoxylin stain were used in selected cases to demonstrate changes in muscle fibers. Where specifically indicated in order to evaluate changes in connective tissue, Verhoeff's elastic tissue stain counterstained with van Gieson's stain was employed. McCallum's Gram stain was used to demonstrate the presence or absence of bacteria. Blocks from selected specimens were stained with sudan III to determine whether fatty degeneration was present.

Anatomic Observations

Gross findings

The hearts usually were moderately increased in weight, as shown in Table I. No significant changes in color or consistency at the time of necropsy were recorded.

Slight to moderate dilatation of the ventricles was found in 24 hearts. Usually only the right ventricle was dilated, but left ventricular dilatation accompanied the more severe degrees of right ventricular dilatation. Significant ventricular hypertrophy was found in only 3 hearts.

There were unusual gross findings in 3 cases in which myocarditis was demonstrated microscopically. One heart (case 1) presented a perforation of the posterior wall of the right atrium. The defect measured about 3 by 4 mm., was oval, and had slightly irregular borders (Fig. 1). There was hemorrhage into the surrounding tissue and there were about 100 cc. of bloody fluid in the pericardial sac.

Verrucous endocarditis of the mitral valve was found in another heart (case 12). There were firm, grayish red, slightly irregular vegetations situated along the line of closure of the mitral leaflets (Fig. 2). No old rheumatic stigmas were found.

There was verrucous endarteritis of a patent ductus arteriosus in a third case (no. 13). The vegetations were friable, brownish red, elevated lesions protruding from the pulmonary end of the ductus arteriosus (Fig. 3).

Histologic findings

Myocarditis

Histologic evidence of myocarditis was found in 14 hearts. The myocardial changes varied considerably in extent and severity. Lesions were

somewhat more frequent in the left posterior papillary muscle and the ventricular septum than in other parts of the heart.

In 3 hearts (cases 1, 2, and 3) minimal acute myocarditis was present. Degenerative changes included irregular affinity of the cytoplasm of

TABLE I

*Heart Weights: Comparison with Normal Standards**

Case	Age	Sex	Estimated body weight	Heart weight	Normal heart weight according to sex and age, or sex and body weight
	years		*lbs.*	*gm.*	*gm.*
1	7	M		120	93.3
2	9	M		165	108.3
3	15	M		330	200.6
4	21	M		335	303.5
5	22	M		228	311.1
6	26	M	150	300	294.0
7	36	F	165	222	297.0
8	21	F		235	250.6
9	31	M	140	295	274.0
10	32	F	150	340	272.0
11	44	M	185	383	363.0
12	34	M	165	340	297.0
13	7	F		140	81.4
14	17	M		336	250.9
15	17	M		250	250.9
16	31	F	120	163	215.0
17	3	M		70	64.5
18	3	M		85	64.5
19	4	M		75	74.7
20	5	M		109	83.7
21	5	M		96	83.7
22	10	M		173	130.9
23	10	M		130	130.9
24	12	M		143	157.0†
25	13	F		253	142.5
26	14	M		267	216.1
27	14	F		161	173.8
28	17	M		353	250.9
29	17	M		315	250.9
30	20	M		355	305.3
31	22	M		382	311.1
32	22	M		310	311.1
33	23	F		220	258.5
34	29	M	155	290	304.0
35	30	M	170	370	333.0

* Normal heart weights from Vierordt and Smith.
† Mean normal heart weight.

muscle fibers for eosin, swelling of muscle fibers, loss of striations, cytoplasmic vacuolation, and occasional fragmentation of muscle fibers. Cellular exudate was not prominent, consisting mainly of small, scattered, perivascular collections of large mononuclear cells with occasional plasma cells and lymphocytes. There was moderate congestion and edema of the interstitial connective tissue. The significance of the appar-

ently minimal changes in these specimens is indicated by the heart in case 1, in which a perforation of the right atrium was present. Except near the site of perforation, where there was severe necrosis (Fig. 4), myocardial alterations were minimal.

In 4 hearts (cases 4, 5, 6, and 7) there was acute myocarditis of moderate severity. The predominant finding was the presence of cellular infiltrate, consisting of large mononuclear cells, situated usually around vessels and in the wider bands of interstitial connective tissue. Neutrophils were present in regions of more severe muscular degeneration (Fig. 5). In one heart a few multinucleated cells were seen. Degenerative changes were similar to those described in the preceding group.

In 6 hearts (cases 8, 9, 10, 11, 12, and 13) there was severe acute myocarditis. The most conspicuous findings were complete focal necrosis of muscle fibers and fairly abundant cellular infiltration (Figs. 6 to 12). Usually, severe necrosis of muscle involved only one or two adjacent muscle fibers. These muscle fibers were completely replaced by irregularly staining, disorganized masses of coagulated cytoplasm. Less marked degenerative changes, including vacuolation, fragmentation, and coalescence of the cytoplasm of muscle fibers, and karyolysis, were found to involve wide regions of myocardium. In the foci of severe muscular degeneration neutrophils usually were abundant. Fairly frequently, however, regions of severe necrosis were found with very little cellular infiltration in their immediate vicinity. In general, the cellular exudate was most prominent around the smaller vessels and in the wider bands of interstitial connective tissue. In these regions large mononuclear cells were numerous. Plasma cells and lymphocytes were less abundant. Congestion and edema of the interstitial connective tissue usually were conspicuous. Frequently the collagenous fibers of the interstitial connective tissue showed an increased affinity for eosin. There was straightening of collagen fibers and a tendency of fibers to form small clumps. This change was most marked around medium-sized vessels.

One heart (case 14) was classified separately as healed myocarditis. The outstanding change was the presence of large regions in the posterior wall of the left ventricle containing practically no muscle fibers (Figs. 13 and 14). In these regions the cellular components consisted mainly of large mononuclear cells situated in a rather delicate lacework of connective tissue fibers, suggesting that extensive complete necrosis and absorption of muscle fibers had occurred, leaving the myocardial stroma and a few macrophages. No vascular lesions were found in association. The remainder of the cardiac muscle showed minimal changes, including

vacuolation and hyaline droplet degeneration of cytoplasm, marked variations in cytoplasmic affinity for eosin, and karyolysis. There were a few scattered plasma cells and lymphocytes.

Selected hearts from each of the groups discussed previously, in which cytoplasmic vacuolation was present, showed only minimal deposition of lipid when stained with sudan III.

Endocarditis

Minimal valvular changes were found almost constantly, with and without associated myocarditis. These changes included separation of valvular stroma as if by edema, and foci of highly eosinophilic bundles of collagen (Fig. 15). These findings were somewhat more marked in the aortic and mitral valves than in other valves and were most prominent in hearts showing myocardial changes.

As mentioned previously, in one patient (case 12) there was acute vegetative endocarditis of the mitral valve. The entire valvular stroma was infiltrated with numerous cells, mainly neutrophils and, to a lesser degree, lymphocytes (Fig. 16). The vegetations consisted of irregular deposits of fibrin which were undergoing organization (Fig. 17). No bacteria were seen. Within the valve leaflets there were scattered thin-walled vessels the size of arterioles, indicating previous vascularization of the valve and possibly, therefore, previous inflammation.

In one heart there was a subendothelial circumscribed mass, about 2 mm. in diameter, situated on the inferior aspect of the mitral valve. This lesion consisted of fibroblasts, budding capillaries, deposits of hemosiderin, and recent hemorrhage. Atypical for a healing vegetation, it may have represented a resolving hematoma.

The mural endocardium frequently showed changes in those hearts in which myocarditis was found. These changes included endothelial proliferation, subendothelial edema, and the presence of cellular infiltration, the cell types corresponding to those in the myocardium. There were no significant endocardial changes in those hearts not showing myocardial inflammation.

Endarteritis of a Patent Ductus Arteriosus

As already mentioned, in one case (case 13) acute vegetative endarteritis of a patent ductus arteriosus was observed. The vegetations, consisting mainly of fibrin and leukocytes, were apparently of recent origin, although beginning organization was noted (Figs. 18 and 19). No bacteria were seen. There was moderately severe arteritis at the site of the vegetations, indicated by marked subintimal edema, many neutro-

phils, and slight endothelial proliferation. The left pulmonary artery was uninvolved. There was associated severe acute myocarditis (Fig. 20).

Pericarditis

In one case (case 14) there were left pleuropericardial adhesions and obliterative pericarditis, raising the question of primary pleuritis with secondary pericarditis and myocarditis. Since there was only minimal inflammatory change in the epicardium, it did not seem likely that the pericarditis led to myocarditis. Although the epicardium in other hearts frequently showed inflammatory changes, in no instance were these changes sufficiently severe to indicate that pericarditis was primary.

Histologic Changes in Hearts Not Showing Myocarditis nor Endocarditis

There were 21 hearts in which the histologic findings were not considered significant. All of these showed slight swelling of muscle fibers, and in most there was minimal vacuolation of cytoplasm with some congestion and separation of muscle fibers as if by edema. The latter findings are probably explanatory of the slight to moderate increase in heart weights shown in Table I.

Report of Cases

The following case reports are presented to illustrate certain unusual findings.

Case 1

A white boy, 7 years of age, living in a community in which there was poliomyelitis, was well until August 26, 1946, when he became febrile and vomited a few times. On August 29, headache and pains in his neck developed. He was admitted to the hospital on August 31 because of dizziness and difficulty in walking. On physical examination there was cervical rigidity but no definite evidence of paralysis. The blood pressure was 130/90 mm. of Hg. The cerebrospinal fluid contained 20 lymphocytes and 7 neutrophils per cmm. and the total concentration of protein was 65 mg. per 100 cc. Hot packs were applied to the neck and back, and penicillin was given. About 12 hours after admission, dysphagia, paralysis of the soft palate, and cyanosis developed. The patient was placed in a respirator, after which it was noted that the pulse was thready. Attacks of cyanosis continued but were partially relieved by the aspiration of secretions from the pharynx. On September 1, at about 12:45 a.m., the patient suddenly became cyanotic. The pulse rate rose from 100 to 180 per minute and the axillary temperature to 104.6° F. The patient died at 1:45 a.m. on September 1, 1946, 6 days after the onset of illness.

The anatomic findings in both the medulla and the spinal cord were characteristic of acute poliomyelitis.

The pericardial sac was found to be distended and grayish blue. When

it was opened, free blood was encountered, the estimated amount being about 100 cc. Hemopericardium was due to a perforation of the right atrium, located in its posterior wall to the right of the base of the valve of the coronary sinus (Fig. 1). The defect measured about 3 by 4 mm., was oval and had slightly roughened edges. There was recent hemorrhage into the surrounding epicardium.

The heart weighed 120 gm. Except for marked dilatation of the right atrium and moderate dilatation of the right ventricle, no significant gross cardiac abnormalities were noted. Histologic study showed mainly degenerative myocardial changes. Foci of swollen muscle fibers with loss of striations and fragmentation of individual muscle fibers were present in all sections examined. Near the perforation of the right atrium such areas were larger and the changes slightly more severe than at other sites (Fig. 4). Scattered neutrophils were present in the foci of more severe degenerative change.

There was bronchopneumonia confined to the lower lobe of the left lung. No significant changes were found in the other viscera.

Case 12

A single white man, 34 years of age, had a familial history of diabetes but had been in good health. Following a poliomyelitis outbreak in his home state, on December 6, 1946, sore throat, anorexia, chills, and fever developed. On December 7 he noted difficulty in swallowing and speaking, and shortness of breath. He was admitted to the hospital on December 9. On physical examination cyanosis was evident and there was diminution of the deep reflexes of the upper extremities. Respirations were of a grunting character, preventing adequate cardiac examination. The blood pressure was 180/80 mm. of Hg; temperature, 102.6° F.; pulse, 120; respirations, 25. Leukocytes numbered 20,000 per cmm. of blood. The cerebrospinal fluid contained 45 lymphocytes and 5 neutrophils per cmm. and the total concentration of protein was 30 mg. per 100 cc. There was severe albuminuria, and a few hyaline and granular casts were found in the urine. The urine also showed a trace of reducing substance and the blood sugar was found to be 238 mg. per 100 cc. of blood. Fifteen thousand units of penicillin were given intramuscularly every 4 hours. Ten thousand units of diphtheria antitoxin were administered also. The patient was placed temporarily in a respirator but fought its use. On December 11, after the intravenous administration of a 10 per cent solution of glucose, cyanosis suddenly became more severe and the patient had a generalized convulsion. Use of the respirator was again instituted, but cyanosis continued and the patient died at 7:00 p.m. on December 11, 1946, 5 days after the onset of the illness.

The anatomic findings in both the medulla and the spinal cord were characteristic of acute poliomyelitis.

The heart weighed 340 gm. The right ventricle was slightly dilated. There were multiple, firm, translucent, grayish red vegetations, each measuring 1 to 2 mm. in diameter, near the free edge of the atrial surface of the mitral leaflets (Fig. 2). No gross evidence of old rheumatic

involvement was found. On histologic study, the vegetations were found to consist of fibrin with beginning invasion by fibroblasts (Fig. 17). No bacteria were seen in either the vegetations or the valvular stroma. The mitral leaflets showed marked separation of stromal elements as if by edema and there was extensive infiltration with cells, mainly neutrophils and, to a lesser degree, lymphocytes (Fig. 16). The valvular stroma contained thin-walled vessels the size of arterioles. There were several small masses of degenerating collagen in the tricuspid valve but the other valves did not present significant changes. The myocardium presented marked inflammatory changes, involving mainly the interventricular septum and the posterior papillary muscle. In these regions there were many foci of necrosis of muscle fibers. Fairly numerous cells, mainly neutrophils and large mononuclear cells, were found in the perivascular and interstitial connective tissue, and dense collections of neutrophils were present in the foci of severe muscle degeneration.

There was early bronchopneumonia. No embolic phenomena were observed. No significant changes were found in the other viscera.

Case 13

A white girl, 7 years old, was well until August 2, 1946, when she complained of pains in her legs. Poliomyelitis was known to exist in her community. On August 3 difficulty in swallowing developed and she was admitted to the hospital. Physical examination showed palatal weakness but no other evidence of paralysis. The temperature was 100° F.; pulse, 80; respirations, 20. The cerebrospinal fluid contained 25 lymphocytes and 20 neutrophils per cmm. and the total concentration of protein was 50 mg. per 100 cc. The patient's condition remained apparently unchanged except for the development of tachycardia (140 per minute). At 6:00 p.m. on August 6, the patient rather suddenly went into a state of vascular collapse and, despite the intravenous administration of plasma, died at 1:06 a.m. on August 7, 1946, 5 days after the onset of the illness.

The anatomic findings in the spinal cord and medulla were characteristic of acute poliomyelitis.

The heart weighed 140 gm. The right ventricle was moderately dilated. No other significant gross cardiac abnormalities were noted. There was a small, irregular, brownish red, friable thrombus, about 3 mm. in diameter, projecting from the pulmonary ostium of a patent ductus arteriosus, the lumen of which measured about 2 mm. in diameter at the pulmonary end (Fig. 3).

Histologic examination of the myocardium showed foci of severe degeneration of muscle fibers in the right ventricle (Fig. 20). In these regions there were small groups of infiltrating cells, mainly neutrophils and large mononuclear cells. Other sections of the myocardium showed only minimal inflammatory changes.

The vegetative thrombus in the ductus arteriosus was apparently of very recent origin, consisting mainly of leukocytes held together loosely by fibrin. Beginning invasion by fibroblasts was noted along the area of attachment to the vessel wall (Figs. 18 and 19). No bacteria were seen in either the vegetation or the wall of the ductus. There was moderately severe superficial arteritis, indicated by subintimal edema, many neutrophils, and slight endothelial proliferation. The left pulmonary artery was uninvolved.

No embolic phenomena were demonstrated. Except for beginning bronchopneumonia, no significant changes were found in the other viscera.

Correlation of Clinical and Anatomic Findings

Age

The youngest patient found to have myocarditis was 7 years of age; the oldest was 44 years. The average age of the patients who had myocarditis was 23.0 years in contrast to the average of 15.3 years of the patients who did not have myocarditis. The average age of the patients who had severe or moderately severe myocarditis, acute or subacute, was 26.5 years, whereas the average age of the patients who had minimal myocarditis was 10.3 years. It appeared that older patients were more often subject to myocarditis than young patients and that the severity of myocarditis tended to be greater in proportion to age.

Sex

The over-all incidence of myocarditis was greater in male patients, with a ratio of about 2.5:1. Since there were, however, only 8 females in this series of 35 patients, the relative incidence of myocarditis was actually somewhat greater among females. It is of interest that both of the 2 pregnant women in this study (cases 7 and 10) were found to have myocarditis.

Duration of Illness

The patient with myocarditis who had the longest survival after onset of symptoms lived 57 days (case 14). The myocardial lesions in this case were classified as healed. The remaining 13 patients who had myocarditis lived less than 11 days after onset of symptoms, averaging 5.2 days of illness. This is only slightly more than half of the average length of illness (9.5 days) of the patients who did not have myocarditis, but since 2 of the patients without myocarditis had relatively long survivals (30 and 60 days, respectively) the importance of this comparison is questionable.

Neurologic Findings

Bulbar symptoms were present in 8 of the 14 patients who had myocarditis and in 14 of the 21 patients who did not have myocarditis. Five patients had severe, generalized paralysis, including symptoms of bulbar involvement. Three of these had myocarditis and 2 did not have myocarditis. Thus there seemed to be no correlation of either bulbar or severe generalized paralysis with the presence of myocarditis in this group of cases.

Cardiac Findings

Since respiratory distress in cases of poliomyelitis may result from involvement of either the spinal cord or the medulla, it was not surprising that dyspnea and cyanosis *per se* were found to be of little apparent value in the diagnosis of myocarditis. Among the 14 patients who had myocarditis, dyspnea had been recorded as a symptom in 12 and cyanosis in 11 patients. Among the 21 patients who were not found to have myocarditis, dyspnea had been noted in 18 and cyanosis in 13 patients. These findings do not, of course, exclude the possibility that a thorough clinical evaluation of the cardiac function of patients who have acute poliomyelitis, correlated with evidence of neurologic causes of respiratory distress, might disclose some diagnostic value for these symptoms.

Although the presence of pulmonary râles was frequently recorded, they could not be attributed definitely to cardiac failure, since it was impossible to exclude inflammatory congestion and the edema which frequently accompanies encephalitis. No peripheral edema was observed.

Precordial pain was present in only one patient. This patient was found not to have myocarditis.

The presence of cardiac murmurs was indicated in the clinical records of 3 patients, each of whom was found to have myocarditis (cases 3, 4, and 10). In each instance the murmur was at the base of the heart. In one patient it was transmitted to the cardiac apex. In another patient (case 7) the heart tones were muffled and the apex of the heart was displaced to the left. Since this patient was pregnant, the latter finding was of questionable significance. This patient was the only one in the series in whom severe bradycardia (44 per minute) was noted. Her case is one of those in the group with moderately severe myocarditis.

Tachycardia was commonly observed, but the incidence and severity of this finding were about the same in the group of patients without myocarditis as in the group with myocarditis. Irregularity of pulse was noted in 2 patients, only one of whom (case 4) had myocarditis.

Cardiac lesions were suspected clinically in 2 patients, one of whom

had a congenital fusion and fenestration of the cusps of the aortic valves but no myocarditis. The other patient (case 3) had myocarditis.

In none of the cases in this study was there a past history or other clinical evidence of rheumatic fever.

Suddenness of Death

Six patients died rather suddenly, 3 of whom (cases 1, 10, and 14) were found to have myocarditis. Except in case 1, in which a perforation of the right atrium was found at necropsy, it was impossible to determine whether or not myocarditis had actually caused sudden death, since bulbar involvement with poliomyelitis was present in each case.

A general correlation of clinical and anatomic findings is presented in Table II.

Etiology and Pathogenesis

Before one ascribes the production of myocarditis to the virus of poliomyelitis it is necessary to consider, as has been indicated by Saphir, other possible etiologic factors, such as bronchopneumonia, serum reactions, and sulfonamide sensitivity, each of which has been reported to have caused myocarditis.

Bronchopneumonia was present in only 4 of the cases in which myocarditis was found, and in none of these patients was the pneumonia severe. It seemed unlikely that bronchopneumonia was of much significance in the pathogenesis of myocarditis.

Although myocarditis has been produced experimentally by the injection of horse serum and has even been reported in a few cases in which human beings have been treated with horse serum, this factor did not seem important as a cause of myocarditis in this group of cases. Only 3 patients who had myocarditis (cases 4, 9, and 12) had received horse serum, and in each the injections were begun 2 days or less before death. None showed clinical evidence of sensitivity to serum.

As has been shown both experimentally and clinically by French and Weller, sulfonamide compounds may cause myocarditis. In the present study 4 patients who had myocarditis (cases 7, 8, 10, and 14) had received a sulfonamide compound, in each instance sulfadiazine. Three of these were given the drug for 2 days or less. There was no associated clinical nor other histologic evidence of toxicity. Administration of sulfonamide compounds seemed of minimal importance as a cause of myocarditis in this series of cases.

The effects of anoxia and general toxemia were impossible to assess adequately. It is probable that these factors may have caused at least some of the minimal cardiac changes observed in the cases in this study.

The reported occurrence of myocarditis in other virus diseases, in-

TABLE II

Correlation of Clinical and Anatomic Findings in 14 Patients Who Had Significant Cardiac Lesions

Case	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Age, years	7	9	15	21	22	26	36	21	31	32	44	34	7	17
Sex	M	M	M	M	M	M	F	F	M	F	M	M	F	M
Approximate duration of illness, days	6	6	7	3	4	10	4	3	6	5	4	5	5	57
Type of paralysis	Bulbar	Bulbo-spinal	Spinal	Spinal	Spinal	Bulbar	Bulbar	Spinal	Spinal	Bulbo-spinal	Bulbar	Bulbo-spinal	Bulbar	Spinal
Dyspnea*	3	2	3	2	3	3		3	2	3		3		3
Cyanosis*	3	3	3		3	3	2	2	3	3		3		3
Oral temperature, °F.†	103	101	102	100.5	102.5	101	102	103	102.5	101	102	101	100	100
Pulse rate, beats per min.†	130	90	120	120	110	80	125	110	80	95	100	110	120	120
Respiratory rate, per min.†	35	33	20	36		20	30	22	30	30		20	30	20
Cardiac murmurs			Basal and apical system	Pulmonary system					Basal system					
Leukocyte count, per cmm. of blood		20,600	9,900			10,600	9,800	11,400	22,700				20,500	14,600
Myocarditis*	1‡	1	1	2	2	2	2	3	3	3	3	3	3	3
Valvulitis*												3		
Endarteritis of a patent ductus arteriosus*													2	
Pneumonia*	2								1			1	1	

* Severity on a basis of 1 to 4.

† Approximate average during period of hospitalization.

‡ Even though myocarditis was minimal in case 1, there was a perforation of the right atrium.

cluding mumps, virus influenza, and epidemic encephalitis, suggests that it is not illogical to consider the virus of poliomyelitis to be capable of causing myocarditis.

Recently Helwig and Schmidt have succeeded in isolating a virus with both neurotropic and cardiotropic features. When injected into mice this virus was shown to cause paralysis of the extremities and myocarditis. These authors did not state the immunologic relationships, if any, of this virus to the virus of poliomyelitis, but this report of a virus disease with features grossly resembling poliomyelitis and with associated myocardial lesions again suggests that it is not unreasonable to suspect the poliomyelitic virus of causing myocarditis.

Since myocarditis was present in 40 per cent of the cases in this series and since other investigators have found an even greater incidence of myocarditis in acute poliomyelitis, it is evident that myocarditis occurs frequently in poliomyelitis. Inasmuch as it has not been possible to explain satisfactorily the etiology of myocarditis in the cases in this series without ascribing it to the virus of poliomyelitis, we are led to the assumption that the virus may invade and destroy cardiac muscle.

The occurrence of endocarditis and ductal endarteritis, such as was observed in cases 12 and 13, is more difficult to relate to the virus of poliomyelitis. Certain proof of the exact etiology of lesions of this type occurring in poliomyelitis, as well as proof of the cause of myocarditis, will depend on the isolation of the virus from such lesions and the experimental production of similar changes.

If it is assumed that the virus of poliomyelitis causes cardiovascular lesions, we are confronted with the problem of the mode of invasion of cardiovascular structures by the virus. It may be of some significance that the virus has been demonstrated in the blood stream of monkeys with poliomyelitis. On the other hand, most investigators believe that the virus migrates along nerves. Evidence of injury to nerves was observed in many of our cases and Saphir and Wile pointed out that nerve fibers of the myocardium were found to be separated by an "edema-like material." Further study will be necessary to evaluate such changes.

It is of interest that cases in which myocarditis was found tended to occur in chronologic sequence. This fact suggests that the poliomyelitis virus manifests marked cardiotropic features in only certain epidemics. This may explain why competent observers frequently have not found evidence of myocarditis.

Comment

Since acute myocarditis is seldom characterized by histologic features which will permit an etiologic diagnosis, it was not surprising that myo-

carditis in this series of cases presented a nonspecific picture. The lesions observed were similar to those which have been described in other infectious diseases and in Fiedler's myocarditis. No Aschoff bodies were found, although there was some degeneration of collagen such as has been described in rheumatic myocarditis. This finding seemed of minimal importance since degeneration of the collagen is rather frequent in the presence of inflammation, regardless of its cause.

It is unfortunate that blood cultures were not made in the 2 cases in which vegetative lesions were found, but the absence of demonstrable bacteria in these lesions is negative evidence in favor of infection by poliomyelitis virus. It is of interest that the lesions in both of these cases involved regions in which there was probably diminished resistance to infection—in one case a patent ductus arteriosus, in the other case a valve which was vascularized, indicating possible previous valvular injury. The absence of such *loci minoris resistentiae* in most patients who have poliomyelitis may partially explain why so few lesions of this character are observed.

The failure of physicians to establish the clinical diagnosis of myocarditis in cases of acute poliomyelitis is not difficult to understand. Myocarditis other than that occurring in rheumatic fever is almost always extremely difficult to diagnose. Moreover, serious neurologic symptoms in acute poliomyelitis have diverted clinical attention from the cardiovascular system. Perhaps a general awareness of the frequency of myocarditis in acute poliomyelitis will facilitate the clinical diagnosis of myocarditis.

Conclusions

Myocarditis occurs frequently in acute poliomyelitis, having been observed in 14 (40.0 per cent) of 35 cases of fatal poliomyelitis.

Acute vegetative endocarditis and endarteritis of a patent ductus arteriosus may occasionally be found in acute poliomyelitis. One example of each of these lesions was present among the cases in this study.

Since the cardiovascular lesions occurring in the cases included in this study were otherwise unsatisfactorily explained, poliomyelitis virus must be considered as a possible cause of such lesions.

Proof that cardiovascular lesions in acute poliomyelitis are caused by the poliomyelitis virus will depend on demonstration of the virus in the lesions and the experimental production of such lesions.

The diagnosis of myocarditis in acute poliomyelitis is seldom made during life. Myocarditis should be suspected in every patient who is seriously ill with acute poliomyelitis.

Myocarditis, as observed in this series of cases, was usually more

severe and proportionately more common in adults than in young children.

The ratio of males to females in the group of patients with myocarditis was 2.5:1, but since the ratio of males to females in the study was more than 3:1, actually a slightly greater proportion of females had myocarditis.

There was no specific correlation of type of paralysis—bulbar or spinal—with the presence or absence of myocarditis.

The actual rôle of myocarditis as a cause of sudden death in acute poliomyelitis could not be determined in this study, except in one patient, who had a perforation of the right atrium. Three of the 6 patients who died suddenly were found to have myocarditis, but all of these patients had bulbar involvement, which might have explained their sudden deaths.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 48

FIG. 1. Case 1. Perforation of the posterior wall of the right atrium. The defect is located just to the right of the ostium of the coronary sinus.

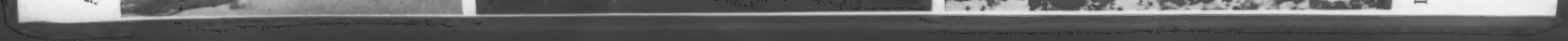
FIG. 2. Case 12. Left atrium and ventricle showing vegetations along the line of closure of the mitral valve. The absence of gross evidence of old rheumatic involvement is apparent.

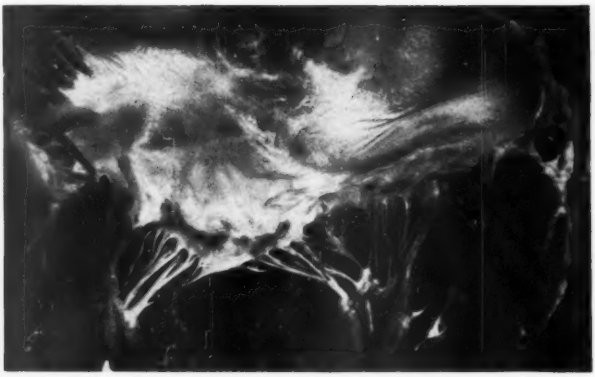
FIG. 3. Case 13. Portion of left pulmonary artery with aortic arch and descending aorta in background. Small vegetative thrombus may be seen at pulmonary ostium of a patent ductus arteriosus.

FIG. 4. Case 1. Right atrium near site of perforation shown in Figure 1. Myocardial fibers have become granular and coalescent. A few neutrophils have infiltrated the area. Hematoxylin and eosin stain. × 580.

FIG. 5. Case 5. Myocardium showing abundant interstitial collections of neutrophils and large mononuclear cells with degenerative alterations of myocardial fibers. Hematoxylin and eosin stain. × 160.

FIG. 6. Case 10. Myocardium with focus of severe degeneration and infiltration of neutrophils. Hematoxylin and eosin stain. × 190.





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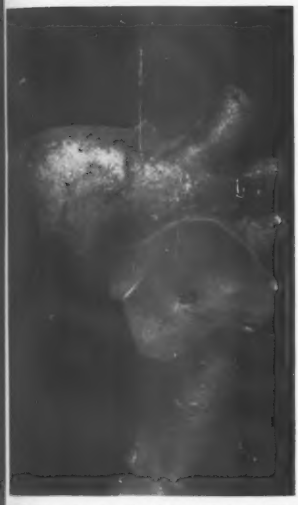

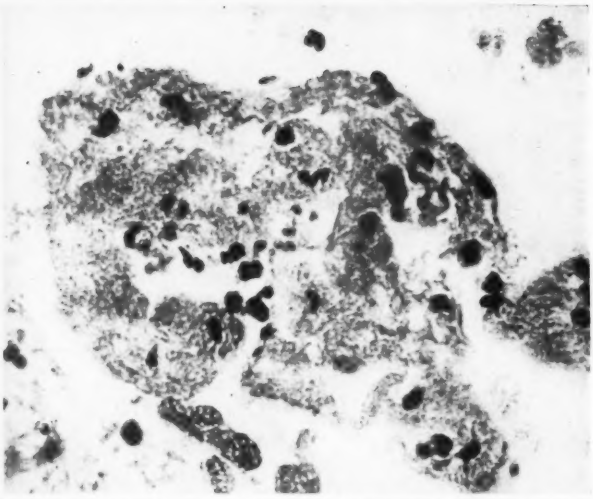

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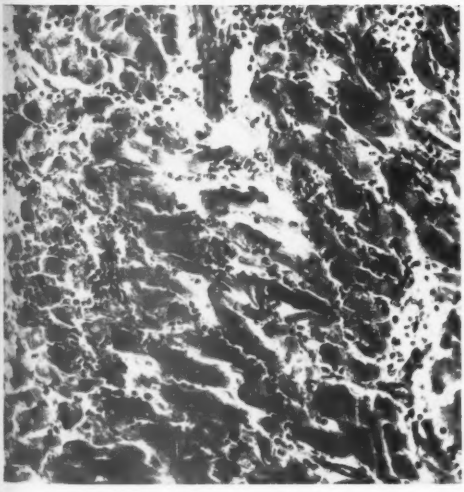

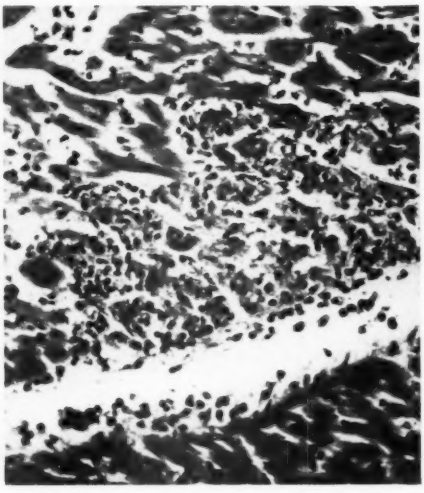

6

Ludden and Edwards Carditis in Poliomyelitis

PLATE 49

FIGS. 7 to 12. The apparent manner of progression of myocardial lesions is illustrated in this series of photomicrographs. Characteristic coagulation necrosis of myocardial fibers is well shown in *Figures 7 and 8* (case 8), stained with Mallory's phosphotungstic acid stain. In *Figures 9 and 10* (case 9) and in *Figure 11* (case 11) infiltration of neutrophils into areas of myocardial necrosis appears. In *Figure 12* (case 12) there is a small area of absence of myocardial fibers. Such a sequence of events probably explains the lesion seen in Figure 13. (Figures 7 and 8 were stained with Mallory's phosphotungstic acid stain; Figures 9 to 12 with hematoxylin and eosin. Figure 7, × 880; Figure 8, × 800; Figure 9, × 1275; Figure 10, × 435; Figure 11, × 600; and Figure 12, × 450.)

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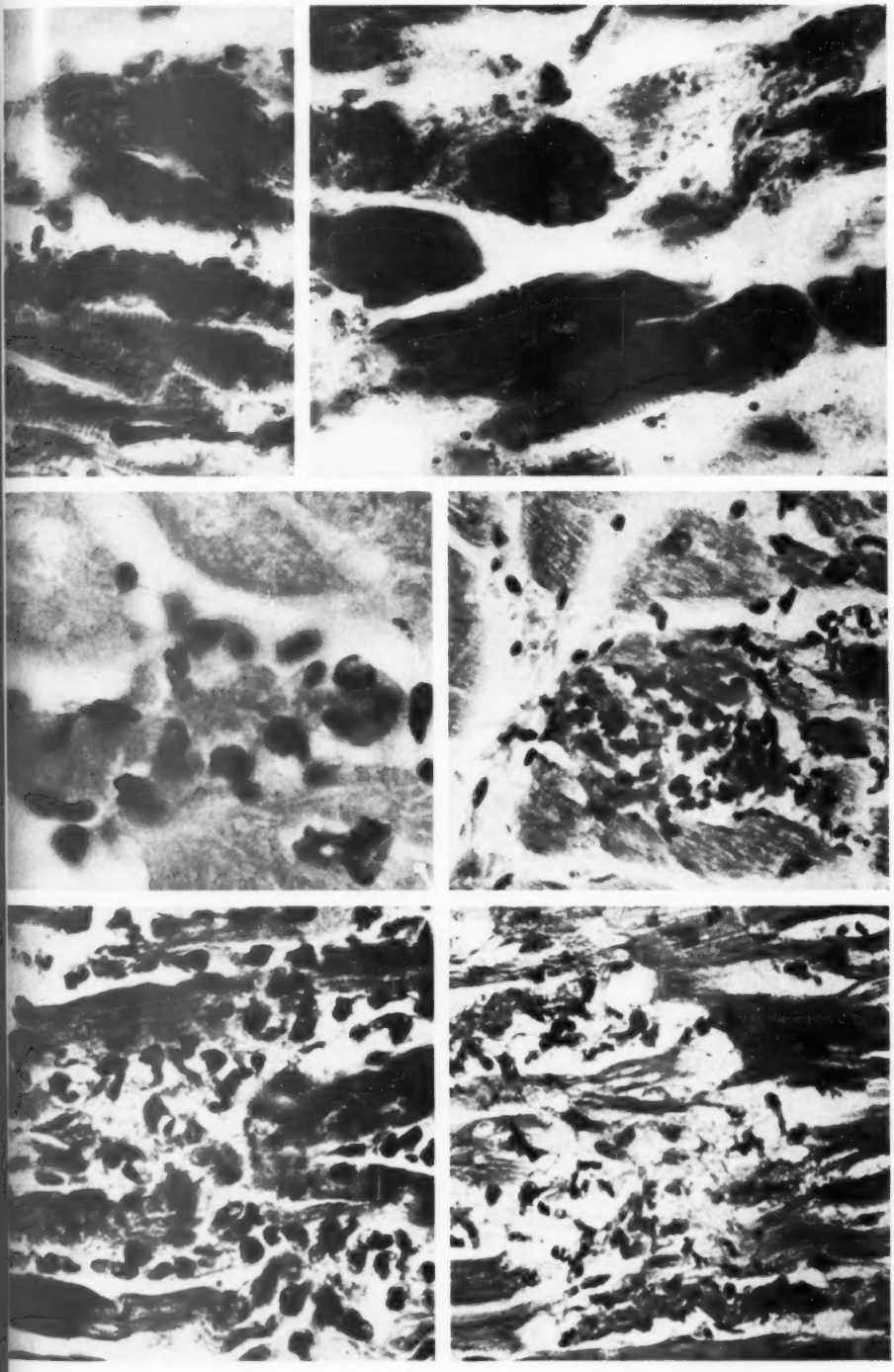
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PLATE 50

FIG. 13. Case 14. Posterior wall of left ventricle. Healed myocarditis, apparently representing the end-result of resolution of an acute process, as shown in Figures 7 to 12. Myocardial fibers have disappeared, leaving only stroma. Hematoxylin and eosin stain. × 85.

FIG. 14. Case 14. Higher magnification of a lesion shown in Figure 13. Lace-like pattern of remaining myocardial stroma is evident. Hematoxylin and eosin stain. × 295.

FIG. 15. Case 8. Mitral valve. Edema of valvular stroma and thickening of collagen fibers, such as were commonly observed with and without associated myocarditis. Hematoxylin and eosin stain. × 260.

FIGS. 16 and 17. Case 12. Mitral valve. In Figure 16 is seen infiltration of valvular stroma with neutrophils. Figure 17 shows an organizing fibrinous vegetation. Hematoxylin and eosin stain. Figure 16, × 800; Figure 17, × 275.

FIG. 18. Case 13. Low-power magnification of a section of the junction of the patent ductus arteriosus and the left pulmonary artery. The lumen of the left pulmonary artery occupies the upper portion of the field. The pulmonary ostium of the patent ductus arteriosus is occluded by a thrombus. Verhoeff's elastic tissue stain counterstained with van Gieson's connective tissue stain. × 11.

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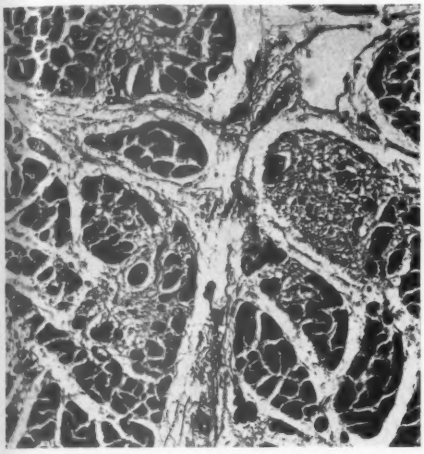

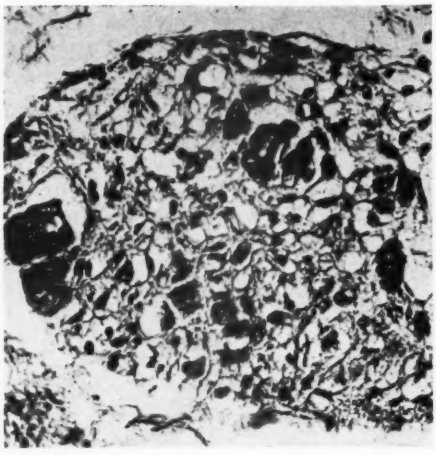

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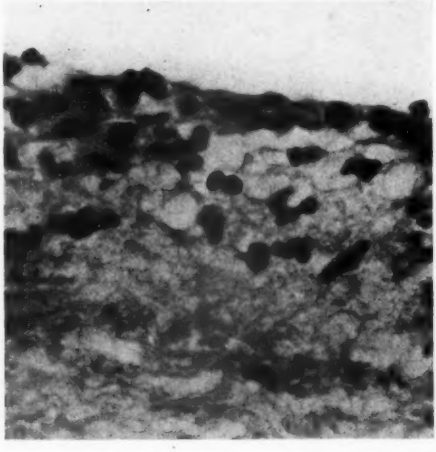

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Ludden and Edwards

Carditis in Poliomyelitis

PLATE 51

FIG. 19. Case 13. Recently formed thrombus and acute endarteritis of patent ductus arteriosus. Subintimal collection of neutrophils. Hematoxylin and eosin stain. × 320.

FIG. 20. Case 13. Severe acute myocarditis. Focal necrosis of myocardial fibers and infiltration of neutrophils. Hematoxylin and eosin stain. × 200.

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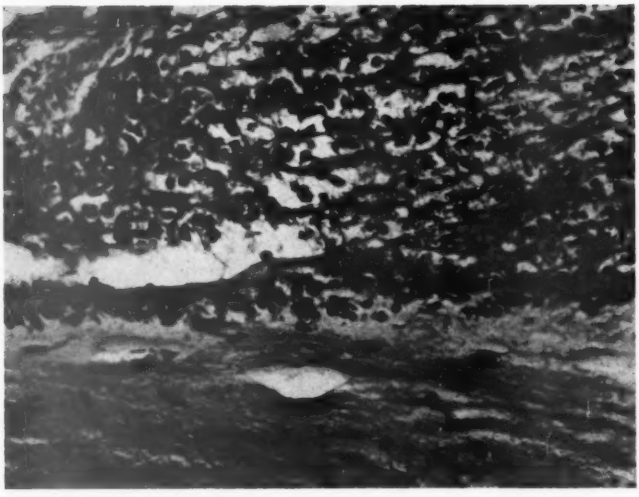

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Ludden and Edwards Carditis in Poliomyelitis

THE FATE OF BLOOD INJECTED INTO THE ARTERIAL WALL *

WILLIAM B. WARTMAN, M.D., and THOMAS C. LAIPPLY, M.D.
(From the Department of Pathology, Northwestern University Medical School and Wesley Memorial Hospital, Chicago, Ill.)

The experiments which are described in this paper were undertaken with the purpose of determining whether hemorrhage in the intima or media of an artery plays a part in the initiation of arteriosclerosis or of dissecting aneurysm. That the intima and media often are richly vascularized in arteriosclerosis and idiopathic medial necrosis has been known for many years, but recently Leary,[1] Nelson,[2] Paterson,[3] Wartman,[4] and Winternitz, Thomas, and Le Compte [5] have shown that hemorrhage from such vascular plexuses may lead to the formation of large hematomas, or of dissecting aneurysms. Such intramural hemorrhages are important because they may obstruct the lumen by their size, or they may precipitate thrombosis. Since surprisingly little is known about what happens to these intramural hemorrhages, it seemed desirable to try to obtain such information experimentally.

METHODS

Healthy, adult bitches were used in all experiments. They were fed purina chow diet and given as much water as they would drink. The common carotid and femoral arteries and the abdominal aorta were exposed with as little trauma as possible, and homologous blood, which was obtained either from the same artery or from an adjacent vein, was injected through a hypodermic needle into the arterial wall. Nothing was added to the blood and the injection was accomplished before clotting occurred. Multiple injections were made in the arteries for a distance of several centimeters. The sites of injection were marked by silk sutures placed in the neighboring tissues. All surgical operations were performed aseptically under nembutal anesthesia.

Later, the arteries were removed at intervals of a few days to many months so that the hematomas could be studied at various ages. Immediately upon removal the involved segment of artery was placed in 10 per cent neutral formalin for 24 hours and then cleared by Spalteholz' method. The areas of hemorrhage were identified easily in the cleared specimens, and blocks were cut of the affected portions and embedded in paraffin. Sections were cut at 4μ and stained with hematoxylin and eosin, Masson's trichrome stain, and a combination of Weigert's elastica and

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van Gieson's collagen stains. The Perls and Turnbull tests were used to demonstrate iron pigment.

Results

It was found that the blood was situated in either the media or the adventitia of the artery but that there was none in the intima. This probably was because the normal intima of dog arteries is extremely thin so that it is virtually impossible to inject significant quantities of blood into it. Thus the results concern principally medial hemorrhages and afford little information about intimal hemorrhages. The effects of the trauma associated with the surgical operations and with the actual injection of the blood could not be separated from the effects caused by the presence of the blood alone.

The results were the same when blood was injected into the carotid arteries, the femoral arteries, or the abdominal aorta; nor were they different in the case of arterial or venous blood. Blood previously withdrawn from the recipient animal was used in all experiments. The resulting hematomas varied in size from 1 to 5 mm.

The changes which were observed in the media will be described briefly, the details being given in Table I.

Eight dogs were used and a total of 26 hematomas were produced in 18 arteries. The hematomas were removed for histologic examination as follows: 1 after 3 days; 4 after 8 days; 1 after 15 days; 1 after 24 days; 1 after 48 days; 5 after 61 days; 1 after 103 days; 4 after 169 days, and 8 after 392 days.

By the third day, organization of the hematoma was already evident and there were many fibroblasts and a few immature capillaries. Nevertheless, the erythrocytes were still well preserved, there was no visible pigment, and the iron stains were negative. Fibrin and moderate numbers of neutrophils were present. There was no demonstrable change in the intima, but the media showed chromotropic degeneration and fragmentation of muscle fibers.

After 8 days the red blood cells were still numerous and appeared well preserved, but pigment was present. This was in two forms. One form was dark yellowish brown, finely or coarsely granular, and stained positively for iron. The other form was very bright yellow, homogenous, and often was precipitated on the elastic fibers. It did not stain positively for iron. Organization of the hematoma had progressed and there were giant cells which had phagocytized some of the iron-containing pigment as well as cellular débris. Fibrin and neutrophils still were present. One of the four arteries observed at this stage showed a hyaline intimal thrombus directly over the hematoma (Fig. 1).

Distinct, well formed capillaries filled with blood first were observed in a hematoma at the end of 15 days (Fig. 3). Despite the presence of these capillaries and an increased amount of iron-containing and non-iron-containing pigment, most of the hematoma was still composed of

TABLE I

Summary of Findings Following Injection of Blood into Arterial Walls

Dog	Artery injected	Age of hematoma (days)	Red blood cells	Iron pigment	Medial scar	Vascularization of media	Intimal plaque
838	Abdominal aorta	3	+++	o	o	o	+
789	Right femoral	8	+++	o	Necrosis	o	o
789	Left femoral	8	+++	++	Necrosis	o	++ Thrombus
873-A	Left carotid	8	+++	++	Necrosis	o	o
873-B	Left carotid	8	+++	++	o	o	o
808	Right carotid	15	+++	++	o	++	o
838	Right femoral	24	o	+++	o	+	++
1380	Right carotid	48	o	o	+	+	o
1379-1	Left carotid	61	o	+++	++	++	o
1379-2	Left carotid	61	o	+	+++	+++	o
1379-3	Right carotid	61	o	++	++	++	o
1379-4	Right carotid	61	o	+++	++	++	o
1379-5	Right carotid	61	o	o	++	+	o
981	Left femoral	103	o	o	+++	o	++
1062	Right carotid	169	o	o	+	o	o
1062	Left femoral	169	o	o	+	o	o
1062	Left carotid	169	o	o	o	o	o
1062	Right femoral	169	o	o	o	o	o
1378-1	Left femoral	392	o	o	++	o	o
1378-2	Right femoral	392	o	o	+++	o	+
1378-3	Right femoral	392	o	o	++	o	+
1378-4	Left carotid	392	o	o	+++	o	o
1378-5	Left carotid	392	o	o	+	o	o
1378-6	Right carotid	392	o	++	+	o	o
1378-7	Right carotid	392	o	o	+++	+	++
1378-8	Right carotid	392	o	+	++	+	o

well preserved red blood cells (Fig. 2). Fibrosis of the hematoma had not occurred. Otherwise the findings were the same as in the arteries examined at the end of 8 days.

After 24 days the red blood cells had completely disappeared and there was a large amount of pigment, most of which stained specifically for iron. Immature fibrous connective tissue as well as fibroblasts and a few capillaries accompanied by mononuclear cells and giant cells were present. No neutrophils or fibrin were observed. One artery showed thickening of the intima over the hematoma by fibroblasts.

The changes were much the same after 48 days, except that there was distinct scarring of the media. Mature collagenous connective tissue, which was well supplied with capillaries, was present.

Vascularized medial scars were still present at the end of 61 days

(Fig. 4). As far as could be determined, the walls of the capillaries were intact and there was no indication that hemorrhage had occurred from any of them (Fig. 5). Small amounts of iron-containing pigment, presumably from the original hematoma, were present. There was no cellular exudate. Occasionally the intima was slightly thickened by the presence of elastic fibers.

The single hematoma which was available for examination at 103 days showed a large, richly vascularized scar in the media and focal fibrosis of the overlying intima (Fig. 6). Neither red blood cells nor pigment were present.

Of 4 arteries observed after 169 days, none showed either red blood cells or pigment. In 2 the vessel walls were restored to normal and only the silk marker remained to indicate where the blood had been placed. In the other 2 vessels there were avascular medial scars.

Eight hematomas were examined after 392 days. Of these, 2 showed complete restitution of the vessel wall so that no evidence of the previous hematoma remained. Medial scars were found in 6 vessels, in 2 of which iron pigment still was present, and in one of which there was a small amount of calcium (Fig. 9). Four of the scars were avascular (Fig. 7) while 2 contained a few capillaries. There was no evidence of hemorrhage from these capillaries. Thickening of the intima over the scar was observed in 4 instances, resulting from proliferation of endothelial cells, collagen, and elastica (Fig. 8).

Discussion

It should be pointed out that neither the formation of atheromas nor dissecting aneurysm was observed in these experiments, and that the new capillaries which grew in the medial hematoma did not bleed. Intimal thrombosis was observed in only 1 of the 18 arteries which were examined and this was but a small platelet and fibrin thrombus. The whole process appeared to be one of organization of the hematoma resulting either in a medial scar or in restitution of the arterial wall. The lesion apparently was self-limited and did not progress to either arteriosclerosis or dissecting aneurysm.

Removal of the blood from the arterial media was accomplished by gradual destruction of red blood cells accompanied by liberation of pigment and of fluid and cellular exudate in which neutrophils predominated. Medial necrosis was present for the first 8 days but was not observed after that time. Red blood cells disappeared between the 15th and 24th days. Hemosiderin pigment was observed first after 8 days and in most instances continued for as long as 61 days. Iron-containing pig-

ment was absent in 4 vessels examined at the end of 169 days, but was discovered in 2 of 8 arteries examined after 392 days. Scars were first seen in the media at the end of 48 days. They were composed of collagenous connective tissue and contained capillaries which tended to disappear between 103 and 169 days, although in 2 animals they were observed after 392 days. Macrophages containing sudanotropic material were not seen at any time.

The significance of the intimal sclerosis which was observed is not clear since a similar lesion was observed in arteries which had not been injected with blood, as well as in untreated control animals (Fig. 10). The sclerotic lesions contained collagenous connective tissue and elastic fibers and sometimes were partly hyalinized, but they were never vascularized nor was atheromatous degeneration observed. Intimal sclerosis was observed 7 times: in one hematoma each after 3, 8, 24, and 103 days, and in 3 hematomas after 392 days.

The occurrence of capillaries in the hematomas is of considerable interest because of the frequency with which they are discovered in the diseased arteries of man. In these experiments they were observed most commonly from the 15th to the 61st day and only a few as late as 392 days, suggesting that the capillaries tended to disappear as the hematoma healed, leaving an avascular scar. They contained blood and always were well formed, with a distinct endothelial lining. They never were observed to rupture and bleed, and thus small hemorrhages such as occur so frequently around the capillaries in an arteriosclerotic artery were completely absent. The capillaries were seen to arise from the vasa vasorum, but in no instance, even though serial sections were examined, were they discovered to communicate with the lumen of the artery, which is another point of difference between the experimentally produced capillaries in normal dog arteries and the naturally occurring capillaries in diseased human arteries.

Summary

Homologous blood was injected into the media of the common carotid and femoral arteries and of the abdominal aorta of normal dogs. Under the conditions of the experiment this blood disappeared within 2 months and the artery either healed, leaving no histologic evidence of the hematoma, or a scar formed in the media. These medial scars were observed as early as 48 days and as late as 392 days after the blood was injected. All of the younger scars were well vascularized with capillaries, but most of the older ones were avascular. The hemorrhages did not enlarge and dissecting aneurysm was not produced. Medial necrosis was seen early but not after 15 days. Although intimal sclerosis was dis-

covered in association with nearly 30 per cent of the hematomas, atheroma did not develop. This intimal sclerosis could not be distinguished from histologically similar lesions which occurred naturally in uninjected arteries and in control animals.

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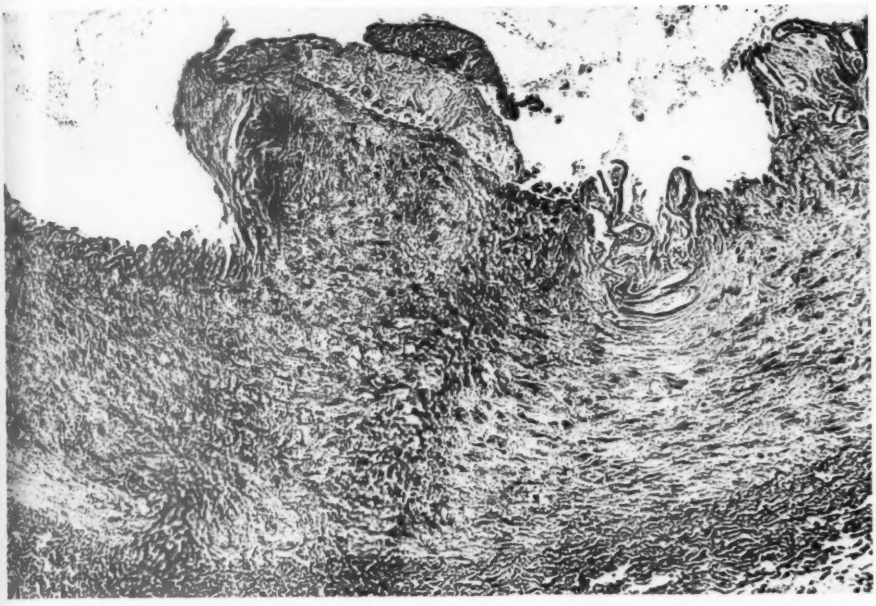
DESCRIPTION OF PLATES

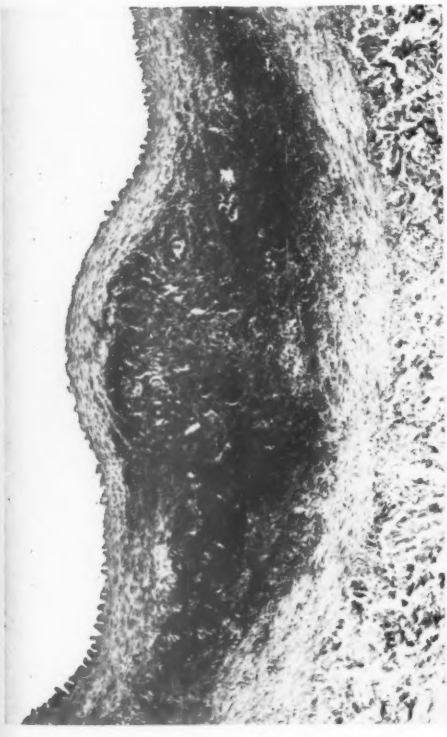
PLATE 52

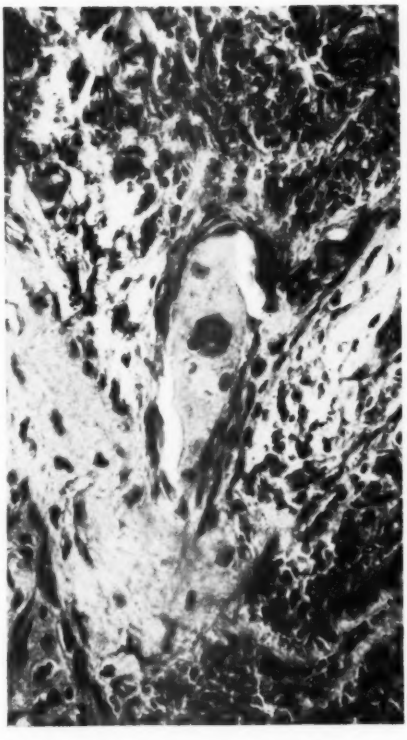
FIG. 1. Dog 789. Fibrin and platelet thrombus covering the intima over the site of injection of blood 8 days after operation. This was the only case in which thrombosis was observed. Hematoxylin and eosin stain.

FIG. 2. Dog 808. Medial hematoma observed 15 days after injection of blood into the right common carotid artery. The wavy black bands at the lateral margins of the hematoma are deposits of blood pigment. Hematoxylin and eosin stain.

FIG. 3. Dog 808. Higher magnification of a portion of Figure 2 showing a well formed capillary. Hematoxylin and eosin stain.







Wartman and Laipply

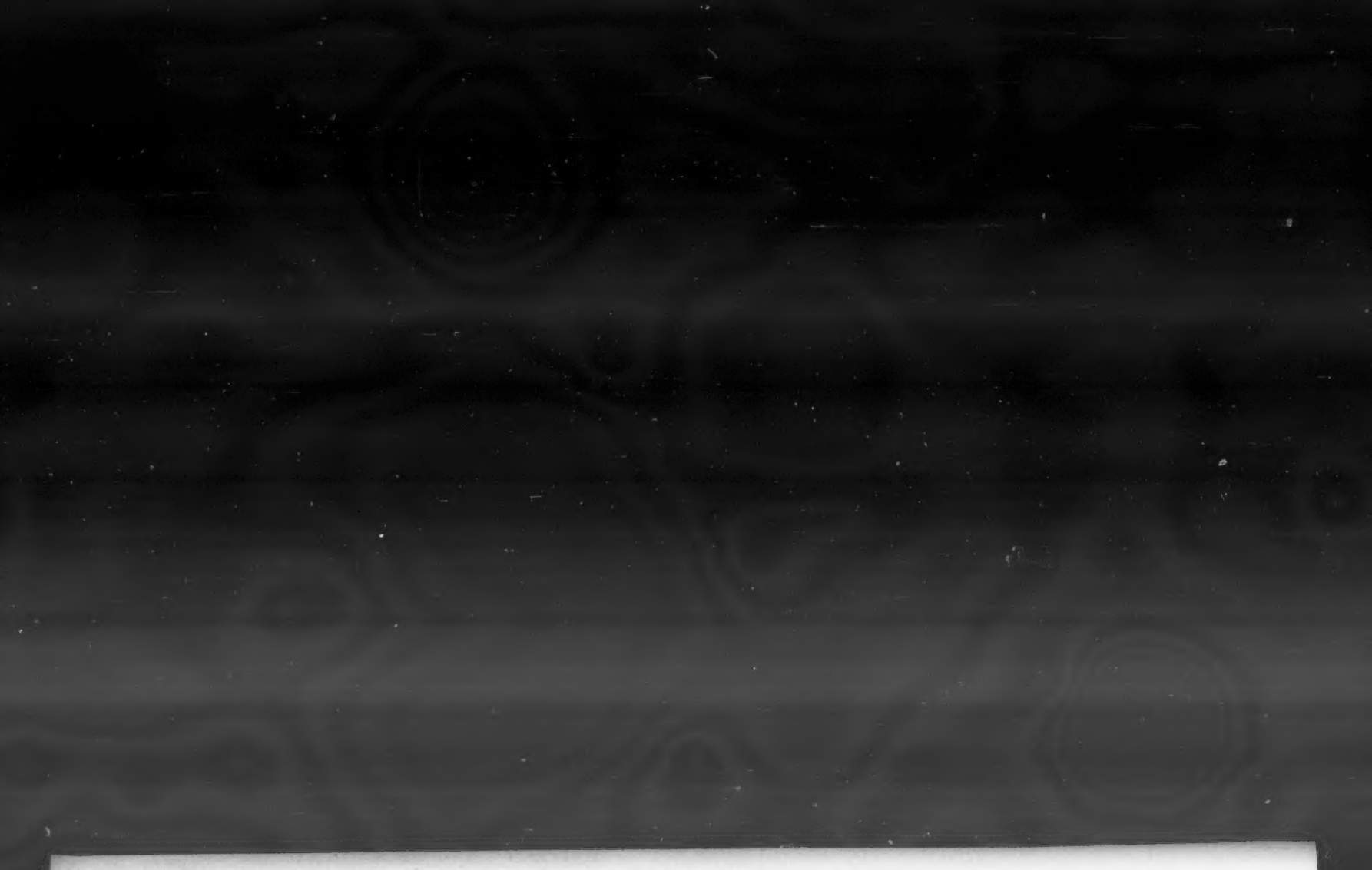
Blood Injected into Arterial Wall

PLATE 53

FIG. 4. Dog 1379-2. A richly vascularized medial scar discovered 61 days after the injection of blood into the media of the left common carotid artery. The intima is normal. Hematoxylin and eosin stain.

FIG. 5. Dog 1379-3. Capillaries in the medial scar of the right common carotid artery 61 days after injection of blood. The intima is normal. Hematoxylin and eosin stain.

FIG. 6. Dog 981. Thickening, fibrosis, and hyalinization of the intima and scarring of the media 103 days after injection of blood into the left femoral artery. There is no atheroma and the internal elastic lamina is intact. Hematoxylin and eosin stain.



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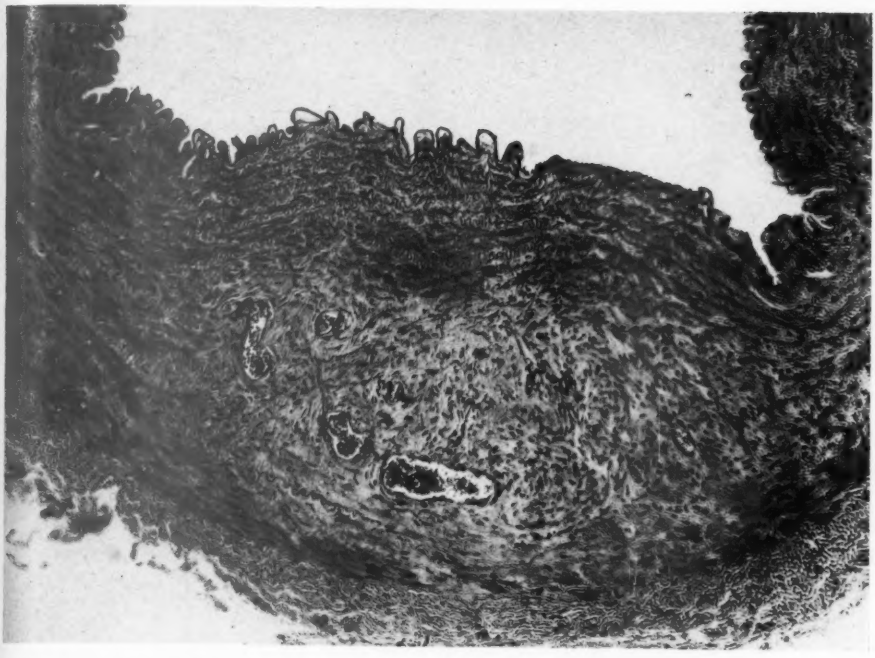

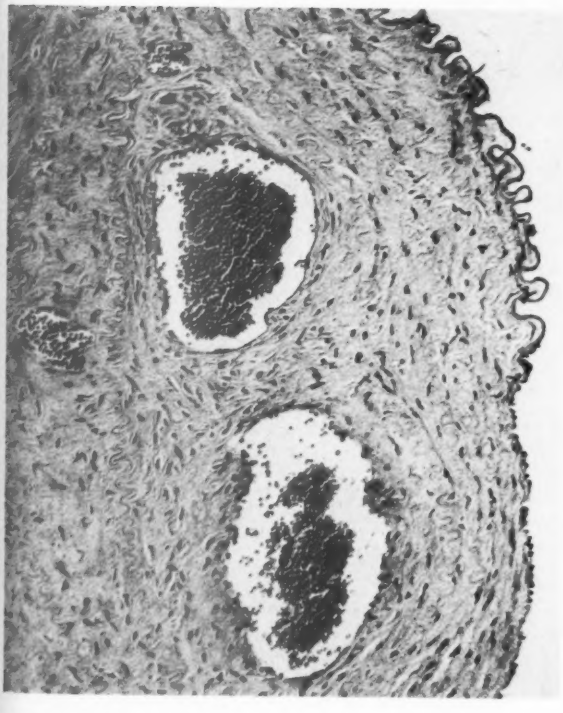

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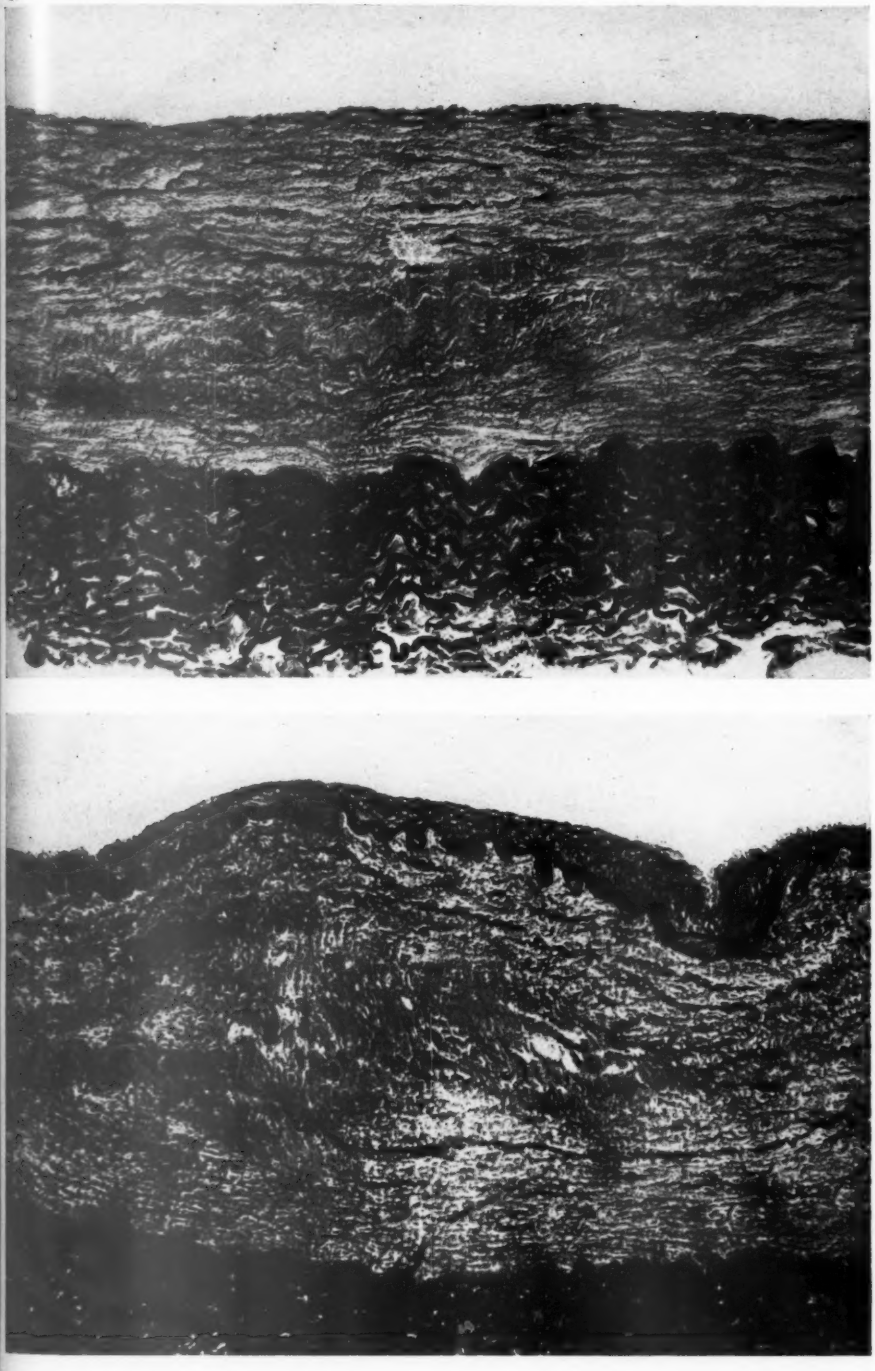
Wartman and Laipply

Blood Injected into Arterial Wall

PLATE 54

FIG. 7. Dog 1378-1. Small avascular medial scar 392 days after injection of blood into the left femoral artery. Van Gieson's and elastica stains.

FIG. 8. Dog 1378-7. Section of right common carotid artery 392 days after injection of blood into wall, showing a medial scar with a few mature capillaries, and intimal fibrosis. Van Gieson's and elastica stains.

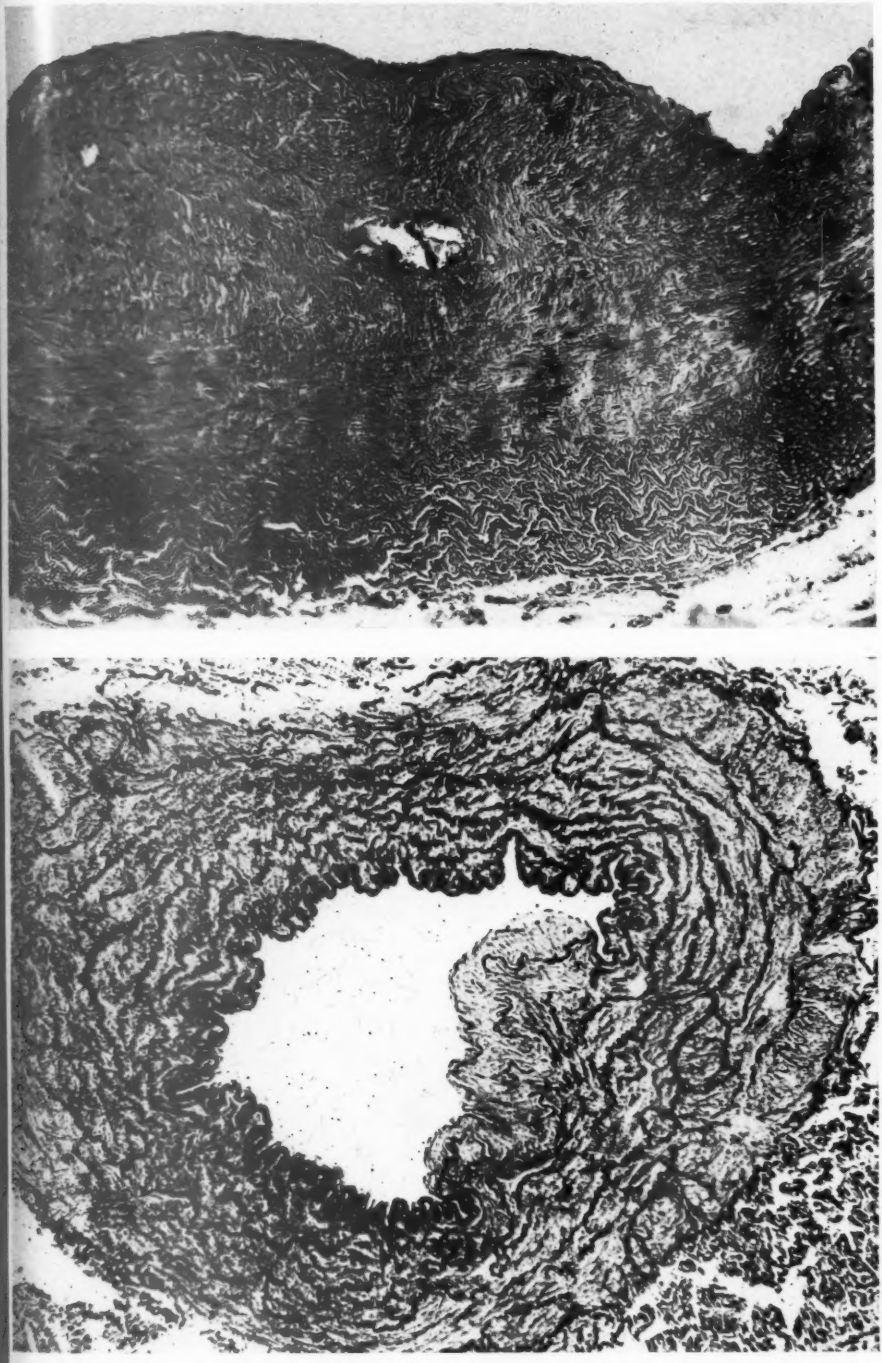


Wartman and Laipply Blood Injected into Arterial Wall

PLATE 55

FIG. 9. Dog 1378. Avascular medial scar with small deposit of calcium 392 days after injection of blood. Hematoxylin and eosin stain.

FIG. 10. Dog 1379. A small artery showing spontaneous intimal sclerosis. No blood was injected into this artery nor was it manipulated. This is thought to be the canine counterpart of human arteriosclerosis, but differs in that atheromatous degeneration usually does not occur. Van Gieson's and elastica stains.



Wartman and Laipply Blood Injected into Arterial Wall

GASTRIC SUBMUCOSAL GRANULOMA WITH EOSINOPHILIC INFILTRATION *

J. VANĚK, M.D.
(From the First Department of Pathology, Charles University, Prague, Czechoslovakia)

In view of the great interest aroused by recent work on the so-called eosinophilic or histiocyto-eosinophilic granuloma (Beck, 1943, Škorpil, 1946), I am presenting several cases showing in the submucosa of the gastric antrum granulation tissue of a peculiar type associated with eosinophilic infiltration.

REPORT OF CASES

Case 1

K. E., a male, 42 years old, had been suffering from "stomach trouble" for some time. He had lost weight and had symptoms pointing to pyloric stenosis. The gastric juice showed normal hydrochloric acid values. Roentgenograms showed pyloric stenosis with a thickening of the mucosa and some gastric retention. Gastric resection (Péan-Rydygier) was performed.

The surgical specimen (no. 6584/44) consisted of the pyloric part of the stomach and was 9 cm. in length. The mucous membrane showed no defect or scar and was freely movable. In the submucosa near the pylorus there was a soft, almost transparent, ill defined node, the size of a small plum, covered with normal mucous membrane. On section it was grayish white to yellow and the cut surface presented many small openings.

Microscopically, the mucous membrane showed atrophic gastritis with islands of metaplastic glands of intestinal type. There was considerable lymphocytic and also a moderate eosinophilic infiltration of the stroma. The lymphatic glands of the lesser curvature showed some hyperplasia.

The node in the submucosa of the pyloric part consisted of cells of the following types (Fig. 5): (1) Fusiform cells with ill defined plasma and oval nuclei poor in chromatin, obviously fibroblasts. Some of these cells were star-shaped. Between the cells a loose network of collagenous fibers could be seen. (2) Irregularly distributed lymphocytes, occasionally accumulated to form rudimentary lymph follicles. (3) Numerous eosinophilic cells of polymorphonuclear type, distributed evenly throughout the pathologic tissue.

The arterioles and capillaries were rather numerous. In addition there were small, round cavities lined with endothelium, obviously corresponding to the openings seen on gross examination. Some of them

* Received for publication, April 21, 1948.

appeared empty while others were filled with a homogenous or foamy substance staining pink with eosin and containing a few lymphocytes. The larger cavities were surrounded by loose fibrous tissue. Undoubtedly all of these cavities were dilated lymphatic vessels.

The relation of the node to the neighboring structures was of interest. The muscularis mucosae was well preserved, in part, separating the abnormal tissue from the mucosa. In places, however, the tissue penetrated between its fibers, and at some points it reached the mucosa. Toward the duodenum it could be traced between the glands of Brunner. In the opposite direction it stopped in the submucosa. Everywhere its borders were ill defined, lacking any tendency to encapsulation. The mucous membrane covering this particular tissue was somewhat thinned out.

Case 2

O. Z., a woman, 64 years of age, had been complaining for about 6 months of pain below the processus xiphoideus, especially during meals. The pain was persistent, not accompanied by belching, and disappeared at rest. There was no hunger pain at night. Vomiting occurred once only, just after a meal. The patient lost 10 kg. of weight. She had not been treated previously. On palpation no resistance or tenderness of the epigastric area was found. Roentgenograms revealed a tumor of the prepyloric part of the stomach. At operation the stomach was found to be freely movable and small. In the prepyloric part a soft, elastic resistance the size of a plum was felt. Resection according to the method of Péan-Rydygier was performed and the patient left the hospital 19 days later in good condition.

In the resected stomach (no. 3991/45), about 2 cm. above the pylorus, there was found a polypoid growth, 2.7 by 1.6 by 1.4 cm. (Fig. 1). It was soft and elastic, and its smooth surface had a brownish violet color. The stalk was 1 cm. long and as thick as a quill.

Microscopically (Figs. 2 and 3), the bulk of the polyp was formed of a peculiar tissue localized to the submucosa. It consisted of fibroblasts and lymphocytes, partly arranged in formations similar to lymph follicles and of numerous, evenly distributed eosinophilic leukocytes. The whole tissue was rich in delicate capillaries and arterioles. Occasionally, cavities with endothelial lining were found, containing a pink substance; they obviously corresponded to lymphatic vessels. The mucous membrane covering the polypus was ulcerated and for the most part replaced by ordinary granulation tissue. The muscularis mucosae was preserved in the stalk only, from which its bundles radiated into the polypoid mass.

Case 3

S. K. was a male, 55 years old, who had contracted a syphilitic infection at age 30 and was treated with neosalvarsan. At about 35 years of age he noticed an indefinite pressure in the epigastrium, especially when his stomach was empty in the afternoons.

He had a feeling of fullness and suffered from belching and sour regurgitation. He often vomited recently taken or partially digested food and bitter-sour fluid. Gastric ulcer was diagnosed, and the complaints disappeared after treatment. Four years before the symptoms had recurred. An operation was suggested but not carried out. A year later he was admitted to the medical clinic owing to a lung complaint. Inflammatory infiltration of the left lung was diagnosed. Roentgenograms of the stomach, taken at the same time, gave the following result: "The stomach showed normal tonus; in the posterior wall of the antrum there was an immovable polypoid formation, the size of a cherry stone. The pyloric passage was free. No retention could be demonstrated, and pressure at the pyloric bulb produced no pain. Three and a half hours after the barium meal, a small remnant was still seen in the stomach while the bulk of the barium had passed into the small intestine." During the patient's further stay at the hospital he did not complain of stomach trouble. On December 10, 1945, the hemoglobin was 105 per cent; erythrocytes, 4,970,000; color index, 1.06; leukocytes, 7,800; differential count: segmented, 55.2 per cent; transitionals, 2.4 per cent; eosinophils, 1.6 per cent; basophils, 0.8 per cent; mononuclears, 8 per cent; lymphocytes, 31.2 per cent; plasmacytes, 0.8 per cent; thrombocytes, normal in number; reticulocytes, 8 per 1,000. Gastric juice (free/total acidity): 32/50, 9/17, 4/13, 30/46, 48/60. The Wassermann reaction was repeatedly positive. Tests for occult blood were positive on several occasions. Blood sedimentation rates were 83, 101, 93, and 103 mm. per hour (Westergren). During treatment with potassium iodide the infiltration of the lung slowly receded and eventually almost disappeared. The patient was then admitted to the second surgical clinic. At that time, the state of nutrition was poor. Percussion of the lungs was normal but there were some ronchi and râles over the left apex of the lung.

In view of the roentgenologic findings, the medical consultants advised operation. A laparotomy was performed under local anesthesia. The stomach was of normal size but there were many thick, flat adhesions, especially on the posterior wall. The duodenum, particularly its bulb, was surrounded and slightly strangulated by firm adhesions which extended to the lower surface of the liver and to the gallbladder. The wall of the latter was not particularly thickened, and no calculi were seen. After separating the adhesions a rigid, localized thickening of the serosa was seen on the anterior wall near the lesser curvature and just above the deformed pylorus. Resection was performed according to Péan-Rydygier. In the submucosa of the prepyloric part a polypoid formation was found.

The postoperative course was uneventful. The patient left the hospital 17 days later. Blood count, taken at that time, revealed: Hemoglobin, 70 per cent; erythrocytes, 3,290,000; leukocytes, 7,600; color index, 1.06; differential count: segmented, 56 per cent; transitionals, 3 per cent; mononuclears, 2 per cent; eosinophils, 1 per cent; basophils, 1 per cent; lymphocytes, 37 per cent.

The patient was seen again 4 months later. He had no complaints, except for persistent coughing. Roentgenograms still showed a shadow over the left apex. Blood counts: Hemoglobin, 65 per cent; erythro-

cytes, 3,200,000; color index, 1.01; leukocytes, 11,800; differential count: segmented, 80 per cent; transitionals, 5 per cent; mononuclears, 0 per cent; basophils, 0 per cent; eosinophils, 0 per cent; lymphocytes, 15 per cent. In the sputum no eosinophilic leukocytes nor Charcot-Leyden crystals were found.

The resected part of the stomach was fixed in a 10 per cent formol solution and submitted for examination. On gross examination (no. 13118/46) no ulcer or scar was visible. In the antrum, 2 cm. from the edge of the specimen, a small polypoid growth was found. Microscopically, the gastric mucosa was practically normal. The polypoid formation was due to an accumulation of abnormal tissue in the submucosa. It had an edematous appearance and was composed of loosely arranged, delicate collagenous fibrils, and of cells with ill defined contours containing elongated, oval nuclei poor in chromatin. Numerous typical eosinophilic leukocytes were distributed in this tissue, particularly in its superficial layers. Lymphocytes, also rather numerous, occasionally showed an arrangement resembling lymph follicles. Besides arterioles and capillaries there were several cavities lined with endothelium. They contained homogenous material staining pink with eosin. The muscularis mucosae covered by the structures described was separated into bundles (Fig. 6), except the most superficial layer, which had preserved its normal parallel arrangement. The peculiar tissue could be traced between the muscle bundles as far as the bottom layers of the mucosa. Toward the surface of the mucosa the eosinophils decreased in number, and the most superficial layer appeared normal except for scattered eosinophils. The pathologic tissue itself showed no sharp lateral delimination from the normal submucosa. As the whole specimen could not be examined microscopically for technical reasons, the mucosa was carefully dissected from the muscularis, but no further foci of a similar appearance were found.

Case 4

A. F., a man, 47 years old, had been in apparent good health until the age of 23, when a left-sided herniotomy was performed. At the age of 45 the same operation was performed on the right side. For 10 years he had been complaining of stomach trouble. Three years previously he reported burning and stabbing pains in the epigastrium, and a gastric ulcer was diagnosed. Seven weeks prior to admission he vomited sour fluid stained with bile. Over a short period he lost 8 kg. of weight. Carcinoma of the antrum was suspected and the patient was admitted to the hospital. The epigastrium was tender to pressure. The gastric fluid had normal acidity. The test for occult blood in the stools was negative. The evening temperature was 37.6° C. without any detectable reason. The Wassermann reaction was negative. Blood counts: Leukocytes, 3,100; erythrocytes, 4,800,000; hemoglobin, 90 per cent; color index, 0.93; differential count: segmented, 53 per cent; transitionals, 5 per cent; eosinophils, 3 per cent; lymphocytes, 39 per cent. Roentgenograms revealed stenosis of the prepyloric part of the stomach, believed to be due to an ulcer in view of the

long history and the normal peristalsis in the stenosed part. Delayed evacuation had not been observed. As cancer could not be excluded, operation was advised.

At operation the pyloric part of the stomach was found to be thickened but there was no sign of a malignant growth. On the lesser curvature typical signs of an ulcer were found. Resection of two-thirds of the stomach according to Billroth II was performed.

On gross examination of the resected part (no. 11/45), a deep ulcer was visible at the lesser curvature about 3 cm. above the pylorus. The stomach wall was considerably thickened and scarring extended as far as the pylorus. Near the edge of the ulcer a grayish white, sharply defined structure the size of a pea was found.

Microscopically, a typical deep ulcer with a calloused base was seen. The edges of the ulcer were partly covered with epithelium. The remaining mucosa was infiltrated by lymphocytes and contained many lymph follicles. The muscularis propria was interrupted by the ulcer, and both its ends were typically raised toward the mucosa. On one side the muscularis propria met the muscular layer of the mucosa, and both were directed toward the scar tissue at the base of the ulcer. On the other side the muscularis mucosae was divided into isolated bundles (Fig. 4). Between these and the muscularis propria a peculiar connective tissue growth was present, thrusting the muscular layers apart and reaching up to the mucosa and to the regenerated epithelium at the edge of the ulcer. From the base of the growth it was separated by a thin layer of connective tissue, rich in collagenous fibrils. It was sharply defined from the muscularis propria.

The structure of this pathologic tissue was almost a replica of that seen in the preceding cases, so that a detailed description appears superfluous. The collagenous fibers, however, were noticeably more numerous, and the cellularity somewhat less.

What makes this case particularly remarkable, however, was the fact that the eosinophilic growth was found in the immediate neighborhood of a peptic ulcer of otherwise trivial appearance. One may question whether the growth or the ulcer was pre-existent. The first possibility cannot be excluded although the ulcer was not situated exactly above the growth. If, however, we accept the view that the latter was secondary to the ulcer, it is necessary to stress the fact that the growth was by no means identical with simple granulation tissue such as is commonly seen at the base of ulcers and may contain a certain number of eosinophils.

Case 5

M. B. was a female, 56 years old, with a history of pneumonia in childhood. At the age of 34 she had icterus which reappeared at the age of 46. For 10 years she had been complaining of pressure in the stomach which usually increased after meals. She had suffered from heartburn and belching, and alternative diarrhea and consti-

pation. About 1 year before these symptoms had improved. For the past 2 years she had felt increasingly short of breath, chiefly at night, and complained of stabbing pain in the chest accompanied by a feeling of anxiety. During the past few years she had lost much weight. On admission, the liver was not palpable. In the middle of the epigastrium a resistance was felt which was only slightly tender to pressure. The patient left the hospital in a somewhat improved condition, but 2½ months later she was readmitted because of recurrence of pain and pressure in the epigastrium. Roentgenograms revealed a polypoid formation of the mucosa in the pyloric part of the stomach. In view of the normal acidity of the gastric juice and the normal blood sedimentation rate, cancer seemed improbable. At operation a movable polyp the size of a cherry, with a thin stalk, was removed from the pyloric part of the anterior wall of the stomach. At the same time the gallbladder containing calculi was removed. Four days after operation the patient developed diarrhea and a sharp rise in temperature (40.5° C.). She died with signs of peritonitis.

On gross examination the resected polyp (no. 896/44) was soft and gray. Microscopically, it was covered with normal mucosa. The bulk of the tumor consisted of a tissue showing the same pattern as in the preceding cases, but for minor differences. The network of collagen fibers was noticeably more dense, and infiltration with lymphocytes and eosinophilic cells was less intense. The muscularis mucosae was dissociated into small bundles at the whole periphery of the polypus by the pathologic tissue, which penetrated into the basal layers of the mucosa.

Autopsy (no. 1505/44) revealed a diffuse purulent peritonitis due to infection with *Staphylococcus pyogenes aureus*. In addition to the artefacts produced by the operation, early osteitis deformans of the skull and slight senile atrophy of the brain were found. No further tumor could be demonstrated in the stomach.

Case 6

P. A. was a female, 56 years old. For the previous 3 months she had complained of frequent spasms of pain in the gastric region, which radiated into the back and under the left scapula. The patient had lost 14 kg. of weight. Physical examination showed tenderness in the epigastric region, where a painful, movable mass was palpable, extending from the borderline of the enlarged liver to 3 fingerbreadths above the umbilicus. Roentgenograms revealed a hypotonic stomach. A dish-shaped, filling defect, 2.5 by 1 cm. wide, with sharply defined borders, was seen. This was situated in the lesser curvature, immediately above the angulus, and close to the anterior wall. The folds of the mucous membrane in the vicinity of the defect were somewhat roughened. Passage through the pylorus was free. A tumor and hypotonic elongation of the stomach were diagnosed.

At operation a tumor was found in the angular part of the lesser curvature, protruding into the lumen. A Péan-Rydygier resection was performed. After the operation the patient developed vomiting and diarrhea followed by dehydration and circulatory collapse. She died 2 days later.

On gross examination the surgical specimen (no. 12811/45) showed a tumorous mass the size of a small walnut, protruding above the level of the mucosa and sharply defined against the muscularis. It was yel-

lowish and soft. Microscopically, the tumor was composed of collagenous fibers with numerous fibroblasts and fibrocytes. Typical eosinophilic leukocytes were evenly spread over the whole tissue but their number was much less than in the preceding cases. Some lymphocytic infiltration was seen also, sometimes forming rudimentary lymph follicles. Occasionally, isolated plasmacytes were found. There were many capillaries and arterioles surrounded by concentrically arranged collagenous fibers and dilated lymphatics were seen as in the preceding cases. The tissue described was situated in the submucosa. It was separated from the muscularis propria by a thin layer of a rather dense and acellular fibrous tissue, and at the lateral borders the collagenous fibers of the submucosa took a course parallel to the surface of the tumor, thus forming a kind of capsule. Toward the mucosa the delimitation was less definite, the muscularis mucosae being separated into single bundles. The mucosa covering the whole area was much thinned out by superficial ulceration, and rather heavily infiltrated with inflammatory cells.

The remaining gastric mucosa showed severe chronic gastritis with widespread metaplasia of the glands to the intestinal type.

At autopsy (24 hours after death) no signs of peritonitis were disclosed. The remaining parts of the stomach contained no tumor. The mucous membrane of the small intestine was covered with mucus.

Discussion

In all 6 cases a peculiar lesion was found in the submucosa of the stomach. Histologically, it consisted of the following elements: (1) Basic connective tissue composed of mesenchymal elements, *i.e.*, fibroblasts or fibrocytes, and loosely arranged collagenous fibers. In the first 3 cases this tissue showed much edema. (2) Infiltration with eosinophilic leukocytes and lymphocytes, the latter being occasionally accumulated in rudimentary lymph follicles. (3) Arterioles, blood and lymph capillaries.

Clinically, all of the cases have many features in common. In case 1 the growth caused symptoms of stenosis and had to be removed by resection. In case 2 the pain under the processus xiphoideus, occurring especially after meals, was most probably due to the pull exerted by the stalk of the polyp which was seated 2 cm. above the pylorus. The third patient had been complaining for 20 years of symptoms suggesting a gastric ulcer, but in the resected stomach no ulcer was found. In case 4 a calloused ulcer had narrowed the gastric antrum, and the growth in the submucosa at the border of the ulcus may have been aggravating the stenosis. Case 5 was complicated by the presence of cholelithiasis and chronic cholecystitis, but the patient's complaints may have been due, at least partly, to the tumorous proliferation in the submucosa of

the gastric antrum. The sixth patient complained for 3 months of recurring abdominal pain which was obviously caused by the growth in the stomach. It is therefore evident that the process described is by no means a mere accidental finding, but may cause serious symptoms requiring operation.

The gross appearance of the lesion was similar in all 6 cases, although there were some minor differences. The tumor-like tissue was situated mainly in the submucosa, forcing the mucous membrane upwards either as a flat prominence (cases 1 and 6) or in the form of a pendulous polyp (cases 2, 3, and 5). In case 4, which is exceptional in this respect, it was situated at the border of a calloused ulcer. On section the tissue was grayish yellow and soft, having an edematous appearance (cases 1 to 3), or rather whitish, firm, and elastic. In some cases (case 1) the cut surface was studded with small openings which turned out to be dilated lymph vessels.

The microscopic findings in individual cases, though having the features in common as given above, showed variations, which, however, were largely quantitative. The differences concerned the amount of collagenous fibrils and the proportion of infiltrating cells. As a matter of fact, these 6 cases have not been grouped chronologically, but according to these differences. Thus in the first 2 the tissue in question was very loosely built, the collagenous fibrils being scarce, the nuclei of the fibrocytes for the most part vesicular, and the migratory cells, both eosinophils and lymphocytes, fairly numerous. On the other end of the series are cases 5 and 6 with much greater quantity of fibers, more mature fibrocytes, and a smaller number of migratory cells. The remaining 2 cases take an intermediate position.

To evaluate these differences it is essential to attempt a classification of the process. This may be considered as an inflammatory lesion of chronic granulomatous character, or as a neoplastic process, *viz.*, a fibroma, with a secondary inflammatory reaction. If we accept the first point of view, it is clear that the differences may be explained on the basis of the different ages of the process, the highly cellular lesion poor in collagen being the more recent one. If, however, we regard the pathologic new-formation as a fibroma, the differences in cellularity would correspond to various degrees of maturity of the neoplasm.

Personally, I am inclined to consider the process as a granuloma. However, the fact should be stressed that it is by no means identical with the "eosinophilic granuloma," such as is found in bones, and may also occur in soft tissues (Škorpil). There are two main differences between these two lesions, not to speak of localization. In the eosino-

philic granuloma the basic cells are not fibroblasts as in my cases, but histiocytes or reticulum cells. Furthermore, the eosinophils in the first process are densely accumulated in some places while in others they are lacking, whereas in my cases they are evenly distributed throughout the lesion.

To avoid confusion, I therefore suggest—pending a better denomination—the somewhat clumsy term "gastric submucosal granuloma with eosinophilic infiltration" for the lesion described. However, I am aware that in some cases, in which the infiltration with migratory cells has greatly receded, the differentiation from a true fibroma may be rather difficult. In such a case even the criteria given by Hueck may be of little help. In fact, I venture to say that some of the alleged fibromas of the stomach actually were of inflammatory origin.

It is obvious that this question cannot be settled satisfactorily unless the etiology of the lesion is cleared up. In this respect, however, very little can be inferred from my own cases, except that the outstanding eosinophilic infiltration of the pathologic tissue is suggestive of some allergic phenomenon.

Kaijser, in 1937, reported 3 cases of allergic disease of the alimentary tract. Of these, the third case is of special interest as the lesion found in the stomach was studied microscopically. The patient was 53 years old and since his youth had been known to be allergic to onion; he had vomited after eating the smallest quantity. One of his brothers had suffered from attacks of migraine, and one aunt had bronchial asthma. At 23 years he had contracted a syphilitic infection, but the Wassermann test later became negative. He had since received no antisyphilic treatment. For 12 or 13 years he had been complaining of gastric trouble, especially in the spring. The diagnosis of gastric ulcer was made and he was repeatedly treated by diet. The gastric trouble increased during the last 18 months. He complained often of hunger pains which disappeared after a meal, but no hunger pains during the night were reported. Roentgenograms suggested an ulcer of the lesser curvature, about 3 cm. above the pylorus. At operation the pylorus was found to be largely adherent to surrounding structures and the serosa was thickened and scarred. A resection according to Billroth I was performed. A blood count taken 9 days after the operation revealed 26 per cent eosinophilic leukocytes. The resected part of the stomach showed diffuse thickening of the walls, particularly toward the pylorus, which was caused chiefly by edema of the submucosa. Neither gross nor microscopic examination revealed an ulcer. The submucosa of the pyloric part of the stomach was very edematous. In its superficial layer there was considerable inflammatory

infiltration, consisting of neutrophilic leukocytes, lymphocytes, and very numerous eosinophilic leukocytes. This infiltration was most pronounced at the top of the folds of the mucous membrane and increased toward the pylorus. The muscularis mucosae was split up into single bundles. Between these, there was inflammatory edema and considerable infiltration consisting mainly of eosinophilic leukocytes. In places, the submucosa showed very many dilated lymphatic vessels, some of them filled with eosinophilic leukocytes, lymphocytes, and endothelial cells, which last were swollen and proliferating, so that they obliterated the lumen. There was much less inflammatory infiltration in the deeper layers of the submucosa. The muscularis propria showed very slight inflammatory infiltration composed again of eosinophilic leukocytes which were mainly situated along the vessels.

Through the courtesy of Dr. Kaijser I was able to study one original slide from that case. The splitting up of the muscularis mucosae, as described by the author, was clearly seen, but in addition to this there were fibroblastic nuclei, the number of which definitely exceeded what might be expected in pure edema. It therefore appears that the lesion has some similarity to that found in my cases, although in the submucosa there was infiltration with inflammatory cells only, which is at variance with my own findings.

In view of the allergic factor in Kaijser's case, I have carefully reviewed the clinical histories of my cases, but I could not find anything definite in this respect. Nor did the hemograms show anything particular, the number of eosinophils at most approaching the upper limit of normal values. Pending further observations, I therefore feel that the question of etiology is to be left open.

Summary

In 6 patients who had suffered from stomach trouble of various kinds, a peculiar lesion was observed consisting of more or less loose collagenous tissue with fibroblasts, lymphocytes, and eosinophilic polymorphonuclear leukocytes. The pathologic tissue thus composed appeared as a circumscribed focus in the submucosa, spreading toward the mucosa of the stomach. Macroscopically, it caused a bulging of the mucosa, and in some cases even a polypous formation. In one case it was situated at the border of a chronic peptic ulcer.

The composition varied somewhat as to the proportion of the collagenous fibrils to the migratory cells, particularly the eosinophils. This seemed to be due to a different degree of maturation of the pathologic tissue.

The lesion is apparently a granuloma, which, however, is definitely different from the "eosinophilic granuloma" of the bone or soft tissues. To point out this difference the provisory term "gastric submucosal granuloma with eosinophilic infiltration" is suggested. In view of a case described by Kaijser, an allergic etiologic factor has been considered.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 56

FIG. 1. Case 2. Gross specimen of the pendulous polyp found in the antrum.

FIG. 2. Case 2. Granuloma enlarging the mucosa and infiltrating the mucosa. The dark areas in the granuloma are foci of lymphocytes. × 27.

FIG. 3. Case 2. Higher magnification of the granuloma showing the relatively great number of eosinophilic cells. × 1000.

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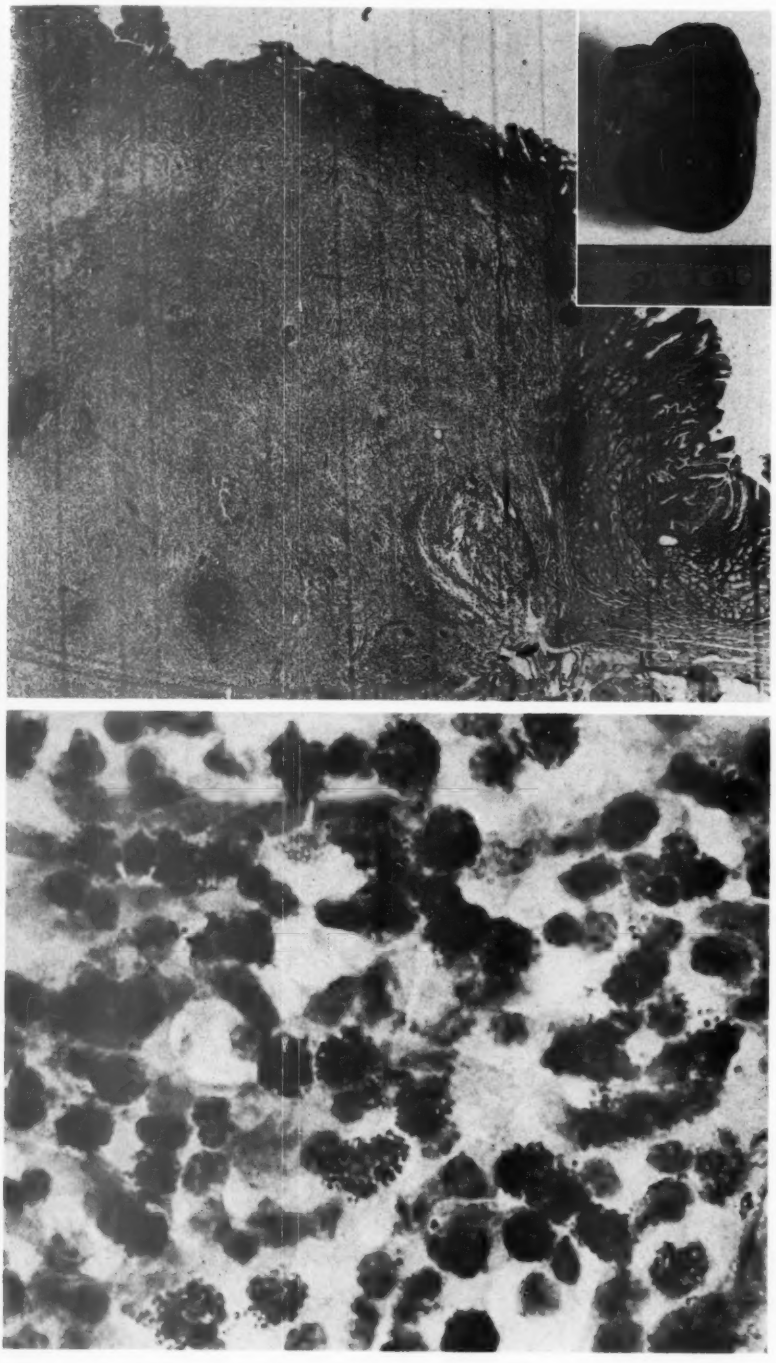

Vaněk

Gastric Granuloma with Eosinophilic Infiltration

PLATE 57

FIG. 4. Case 4. Drawing to show the topographic relations of the granulomatous tissue in the submucosa to the peptic ulcer. The muscularis mucosae is split into isolated bundles.

FIG. 5. Case 1. Typical pattern of the lesion in the submucosa includes fibroblasts with swollen nuclei, eosinophilic leukocytes, and lymphocytes. The lymphocytes are grouped as a rudimentary lymph follicle in the lower portion of the field. Hematoxylin and eosin stain. × 260.

FIG. 6. Case 3. Splitting up of the muscularis mucosae of the stomach into isolated bundles by the granuloma. Heavy infiltration of eosinophils and lymphocytes. Hematoxylin and eosin stain. × 180.

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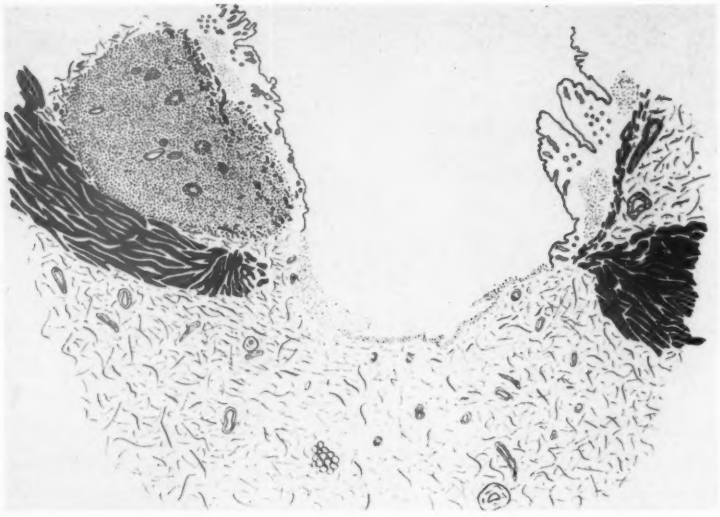

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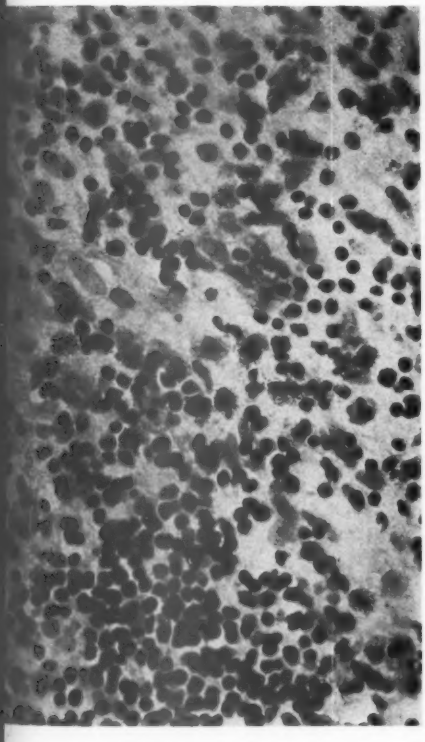

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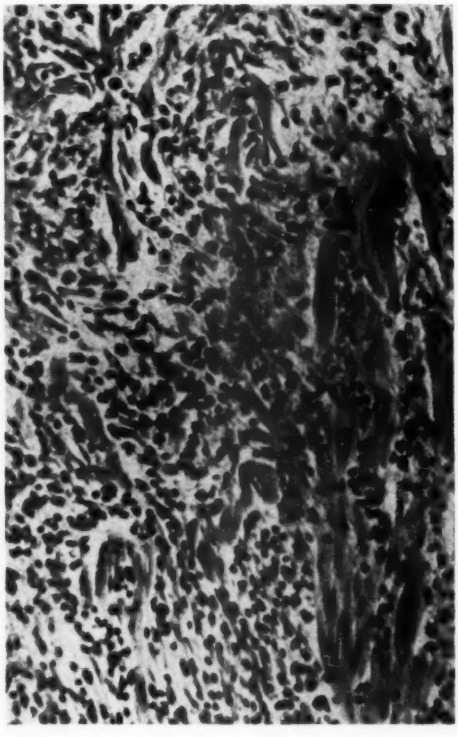

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Vaněk Gastric Granuloma with Eosinophilic Infiltration

LESIONS OF HYPERSENSITIVITY INDUCED IN RABBITS BY MASSIVE INJECTIONS OF HORSE SERUM *

ROBERT H. MORE, M.D., and CHESTER R. MCLEAN, M.D.
(From the Department of Pathology, McGill University, Montreal, Que.)

It is a familiar thesis that hypersensitivity plays a major rôle in the production of the clinical manifestations and histopathologic changes in a number of diseases.[1,2] Many writers have discussed the importance of hypersensitivity in man in the pathogenesis of rheumatic fever,[3-10] periarteritis nodosa,[11-16] Loeffler's syndrome,[17-19] rheumatoid arthritis,[1,2] and other possibly allied conditions,[19-23] and it has been concluded that certain morphologic changes are characteristic of tissue reactions of hypersensitivity.[19,21,23-25]

This conclusion was based on morphologic studies of lesions attributed to hypersensitivity in man [14,15,19,21,25] and those produced experimentally in animals.[4,5,26] The basic changes which were stressed consisted of evidence of increased capillary permeability, edematous swelling and fibrinoid necrosis of collagen, together with a cellular infiltration of variable quantity and type.[21,24,25,27,28] These collagenous alterations and cellular infiltrations were often of focal character and frequently occurred in relation to small vessels, with or without involvement of the vessel wall itself.[21,29]

Klinge[4] and Vaubel[5] were the first to emphasize the similarities between some of the lesions of rheumatic fever and periarteritis nodosa in man on the one hand, and experimentally induced lesions which they attributed to hypersensitivity to foreign proteins on the other. More recently their work has been repeated and extended. Rich has described periarteritis nodosa in man allegedly due to hypersensitivity to sulfonamides[14,15] or to iodine[16]; and Rich and Gregory[8,9,12] have described, in rabbits treated with massive doses of horse serum, lesions which resembled those of periarteritis nodosa and of rheumatic carditis in man.

The importance of an understanding of the pathology of hypersensitivity is heightened by the growing recognition of a variety of conditions that present the vascular damage and the collagenous necrosis which are often considered to be characteristic of the tissue reaction of hypersensitivity. Because of the basically similar morphologic alterations, some authors[19-21,30] have placed in one group some or all of the following diseases: periarteritis nodosa, rheumatic fever, rheumatoid arthritis, dermatomyositis, scleroderma, and disseminated lupus erythematosus. Teilum [21] and Bergstrand [19] believed that in these conditions there was

* Received for publication, May 10, 1948.

a common pathogenetic relationship based on the reaction of hypersensitive tissues. While hypersensitive tissue response may be regarded as strongly suspect in the rôle of the pathogenetic mechanism in these diseases, the evidence is not as yet conclusive and until it is, they are perhaps better regarded as diseases of unknown etiology and pathogenesis.

Two further reasons exist for exercise of caution in accepting hypersensitivity as the pathogenesis for such lesions on the basis of structure alone. First, there is the failure to find in human cases with well established hypersensitivity lesions which consistently possess the collagenous necrosis common to the preceding group of diseases of unknown etiology; and secondly, there is the wide variation in the structure of the lesions in those cases of established hypersensitivity.[14-16,28-32] In the latter group of cases, sulfonamides, iodine, aspirin, and horse serum have been mentioned as antigenic agents. The wide variety of lesions attributed to one or another of these antigens include: a necrotic and proliferative polyarteritis; granulomata, both paravascular and focal in the heart, liver, and kidney; focal necrosis of the myocardium, liver, and bone marrow; massive necrosis of the liver; and tubular degeneration of the kidney. Of these human lesions with the pathogenesis fairly definitely established to be due to hypersensitivity, only the arteritis shows the collagenous necrosis characteristic of the previously mentioned diseases considered by some to have a common pathogenesis. Furthermore, the only experimental lesion of hypersensitivity which consistently has a similar appearance to possible or proved lesions of hypersensitivity in man is the experimental polyarteritis. However, a recent report[33] of a case of periarteritis nodosa of unknown etiology with granuloma, possibly analogous to the periarterial granuloma of sulfonamide hypersensitivity,[29] may indicate a closer relationship than was previously suspected between the human lesions known to be due to a reaction of hypersensitive tissues and the group of diseases of essentially unknown cause mentioned above.

From the experimental side the precise nature of the lesions which can be attributed to hypersensitivity on a morphologic basis is obscured by a considerable lack of uniformity in the reports of hypersensitive tissue reactions in experimental animals.[4,5,8,14,15,30-32,34-37] The issue becomes still more confused by reports that lesions similar to Aschoff nodules and those of periarteritis nodosa occur in rats following the administration of massive doses of desoxycorticosterone acetate.[38,39] Manifestly, these observations cast some doubt on the actual pathogenesis of lesions hitherto attributed to hypersensitivity, both in man and experimental animals.

As an approach to the solution of some of these problems, we have

carried out a series of experiments somewhat more extensive than previously reported, with a view to determining how great a variety of lesions can be produced in different tissues as a result of hypersensitization induced by massive injections of horse serum. These experiments follow in natural succession those of Loncope,[34-36] Klinge,[4] Vaubel,[5] and Rich and Gregory,[8,9,12] but differ from them in that a standard technic of hypersensitization was employed in a much larger series.

Materials and Methods

The rabbits used in this experiment were derived from several sources. The majority were albinos, although several other strains were included. The proportion of males to females was roughly equal. Their average weight was 1800 to 1900 gm. although a few animals as small as 1400 to 1500 gm. were used.

Table I

Relation of Incidence of Arteritis to the Number of Injections of Horse Serum

No. of massive injections	No. of animals in group	No. of animals in group with arteritis
1	6	1
2	38	22
3	7	3
4	4	4
6	9	7
7	6	5
8	7	5
Total	77	47

Whole horse serum was used as an antigen. The Department of Microbiology of the University of Montreal very kindly supplied this after separating the serum from the clot. We then passed it through a Seitz filter and stored it in rubber-capped bottles at 4°C. until ready for use. A preservative was not added to the serum, and numerous test cultures taken immediately before use failed to show bacterial contaminants.

Each animal was given an initial intravenous dose of 10 cc. of serum per kg. of body weight. When successive injections were given, they were repeated every 17 to 18 days. Since the animals gained weight, the absolute dose tended to increase, but was arbitrarily limited to a maximum of 20 cc. per injection. To desensitize the animals partially, and to reduce somewhat the mortality rate from anaphylactic shock, the repeat injections were preceded by 24 hours by an intravenous injection of 1 cc. of serum.

The total duration of the experiments varied from 8 to 146 days. Seventy-seven animals received one or more massive injections (Table I).

Eight died immediately of anaphylactic shock, or within a few hours after their last injection. The remaining animals were sacrificed by air embolism, usually 6 to 10 days after the final injection of serum. Complete autopsies were performed and blocks of tissue were fixed in a 10 per cent saline solution of formol. Histologic sections were prepared from brain, spinal cord, larynx, lungs, heart, aorta, liver, pancreas, stomach, intestines, mesentery, kidney, adrenal, testes, ovaries, bone marrow, thymus, spleen, skeletal muscle, and knee joint, and were stained routinely with hematoxylin and eosin. Where indicated, Masson's trichrome stain for connective tissue, Weigert's stain for elastic fibers, Glynn's stain for bacteria, and the phosphotungstic acid stain for fibrin were employed.

Twenty-five animals, derived from the same source as the treated subjects and living under the same conditions, served as controls. They were not treated in any way. They, too, were killed by air embolism and the same tissues examined as in the treated animals.

Results

Examination of the untreated control animals revealed spontaneous lesions in many organs in a large percentage of animals. Those most frequently encountered were seen in the brain, lung, liver, stomach, intestine, kidney, and testis. The incidence of vascular and focal inflammatory lesions of the brain of the treated and of the control animals was approximately the same as that reported by Bender.[40] Goodpasture[41] has attributed these, as well as a focal nephritis and interstitial pneumonitis, to the organism *Encephalitozoon cuniculi*. The latter lesions were found in about one-fifth of both treated and control animals. Coccidiosis of some degree in the liver of about one-third of both treated and control animals was noted. An unidentified parasite was found in the testis in one instance. Medial sclerosis, sometimes with calcification of the root of the aorta, was noted also in about 30 per cent of the control animals and in 10 per cent of those treated.

The spinal cord, bone marrow, thymus, thyroid, adrenal, pancreatic islet and acinar tissue, and ovaries failed to show any significant lesions in either control or treated animals.

Lesions occurring in the treated animals, but not in the controls, were found in arteries of the heart, lungs, mesentery, stomach, pancreas, liver, and fallopian tubes; in the aorta, and valve rings of the heart, and in skeletal, cardiac and smooth muscle (Table II). Nonspecific inflammatory lesions were found in joints of both treated and control animals, but were more marked in the treated group.

TABLE II

Incidence of Lesions Found in the 77 Animals Injected with Horse Serum but not in Control Animals

Arteries	Coronary arteritis	47	61.1%	Heart	Massive necrosis of myocardium	7	9.1%
	Aortitis	11	14.3%		Lesion in valve ring	10	13.0%
	Pulmonary arteritis	5	6.5%		Lesion in valve cusp*	6	7.8%
	Mesenteric arteritis	4	5.2%	Lungs	Proliferative lesion in vessels	5	6.5%
	Gastric arteritis	3	3.9%		Capillary thrombosis	1	1.3%
	Pancreatic arteritis	4	5.2%	Skeletal muscle	Acute myositis	15	19.5%
	Fallopian tube arteritis	2	2.6%	Smooth muscle	Acute myositis	2	2.6%
	Hepatic arteritis	1	1.3%	Knee joints	Arthritis	6	11.3%†

* Lesions of this character were found also in the mitral valve of 2 control rabbits.
† Knee joints of only 53 animals were examined.

Arteries

Of the 77 animals which received one or more injections of serum, 47 showed arterial lesions. Among these, 16 exhibited inflammatory changes which were classified as acute, 34 as subacute, and 15 as healing or chronic. In every animal in which an acute, subacute, or healing arteritis was found, the coronary arteries were involved. In addition, the pulmonary arteries were so involved in 5 cases, the mesenteric in 4, the gastric in 3, the pancreatic in 4, the hepatic in 1, and the arteries of the fallopian tubes in 2 cases. In no instance did the renal or splenic arteries show a similar inflammatory change. Some arteries showed a striking segmental distribution of the damage (Fig. 3).

While an acute arteritis followed the injection of a single massive dose of serum in 16.6 per cent of cases, the incidence of these lesions rose to 58 per cent with 2 injections and to approximately 73 per cent when 3 or more injections were administered (Table I). There was no significant correlation between the total duration of treatment and the histologic appearances of the lesions.

Acute Arteritis. The slightest alteration seen was a minimal loss of detail in the media of small arteries without any other disturbances in the arterial wall. This was followed by moderate edema with some distortion of the structure of the three coats of the arterial wall (Fig. 1).

In some of these cases there was added to this minimal disturbance an accumulation of mononuclear cells in the intima and adventitia. A more common and severe degree of damage was indicated by a marked edema of the media which separated the muscle bundles widely and which usually was associated with a marked accumulation of lymphocytes, polymorphonuclear leukocytes, and numerous large mononuclear cells in the intima and media. In addition, there was a marked edema of the subendothelial layer of the intima producing a separation of the swollen endothelial cells from the media (Fig. 2). In some cases the edema was so severe as to reduce the myoplasm to thin strands, leaving swollen vacuolated nuclei as the only prominent remaining structure of the media (Fig. 2). The internal elastic lamina became thin, less refractile than is normal, and lost its wavy contour simultaneously with the above changes (Fig. 2). A further degree of damage to the arterial wall was indicated by the presence of varying amounts of a homogeneous eosinophilic material which gave an altered reaction to some connective tissue stains, and stained selectively with those for fibrin. This material was found principally in the media, to some extent in the intima, and rarely in the adventitia. The least alteration of this character consisted of small blobs of this material in the media, and also within the edematous intima. At this stage it was difficult to decide whether, in the media, this material lay between muscle cells, merely filling the edematous spaces described above, or whether it involved the actual cytoplasm of the muscle cells. When a large quantity was present in the media, it obliterated all structures except the prominent swollen muscle nuclei, and in many cases it formed a large homogeneous eosinophilic lake which appeared to contain only the floating remnants of pyknotic nuclei (Fig. 2). A small patch of this material occasionally was present in the adventitia. In these areas a gradual change in the character of a collagenous fiber was seen as it was followed from a normal into a damaged area. This consisted first of a slight swelling and then of a change to deep eosinophilic homogeneous-staining material. The latter alteration may have been due to an alteration of the substance of the fiber or may have resulted from the covering or infiltration of the fiber with material which stained in the above manner. While alterations occurred in the internal elastic lamina as described above, it remained comparatively intact when other structures had been lost in an extensive fibrinoid necrosis. In the midst of such a change the outline of the internal elastic lamina, considerably thinned and straightened, could usually be seen (Fig. 2). However, in some very extensively damaged areas it was broken and there was a fusion of the homogeneous eosinophilic contents of the media and intima

across this break (Fig. 2). The various changes described above often were found in different segments and coats of the same artery and in different arteries of the same animal (Fig. 3).

Subacute Arteritis. The subacute inflammatory reactions in vessels were characterized particularly by proliferative changes of the intimal and adventitial cells, together with a variable amount of cellular exudate consisting mostly of mononuclear leukocytes. Occasionally the leukocytic collections were of paravascular distribution and presented a somewhat granulomatous appearance, which in the heart vaguely resembled Aschoff nodules.

Chronic and Healing Arteritis. In the stage of chronic and healing arteritis the intimal layer was irregularly thickened with cellular connective tissue, the cells of the intimal endothelium were somewhat swollen and hyperchromatic, and the media was slightly distorted because of fibrosis. The adventitia was rendered irregularly thick and dense by the presence of collagenous scar tissue, and in this coat lymphocytes and large mononuclear cells were seen. The comparative resistance of the internal elastic lamina to the destructive forces which damage other tissues of the vessel wall was apparent in the healing stage, in which the outline of the internal elastic lamina could be made out in the midst of fibrous proliferative repair in the adjacent media and intima (Fig. 4). With the loss of edema fluid and cellular exudate, the media in some cases assumed an almost normal appearance (Fig. 5). None of the animals was allowed to live long enough after the last injection to determine to what extent healing of these lesions would occur.

Comparable arterial changes were not seen in any of the 25 control animals.

Aorta

Only the first portion of the aortic arch was examined. In 11 of the treated animals an inflammatory reaction of the intima consisting of edema, cellular infiltration, and swelling of the intimal endothelium was observed. The change was diffuse, involving wide areas of the aortic lining and occasionally extending into the coronary ostia to become continuous with an acute necrotizing arteritis of the coronary arteries. The intimal tissues frequently were edematous and sparsely sprinkled with a mixed mononuclear exudate including lymphocytes, plasma cells, and large mononuclear cells (Fig. 6). In only one case did the media appear to be involved. In that instance, small masses of fibrinoid material, pyknotic nuclei, and nuclear fragments, together with occasional polymorphonuclear leukocytes, were observed in a few areas of the inner one-third. These lesions bore no resemblance to the familiar spontaneous

calcification of the media of the aorta of rabbits which sometimes was encountered among the animals of the experimental group.

These changes of the intima and media were not seen in the aortas of any of the control animals, although they too showed calcification of the media in about the same incidence as the experimental group.

Heart

Inflammatory and degenerative lesions of the myocardium and valve cusps were found in both treated and control animals, and of valve rings and coronary arteries in treated animals only.

The inflammatory reactions involving the coronary arteries have been discussed under the heading of "arteries."

Myocardium. In the myocardium, diffuse and focal collections of lymphocytes together with scarring and proliferation of connective tissue elements were seen in some degree in virtually all of the animals of both treated and control groups. These changes were comparable to the spontaneous myocardial lesions of rabbits so well illustrated by Miller.[42] In the hearts of 7 treated animals, however, there was observed a massive necrosis of large areas of muscle for which there was no counterpart in the control material. The involved muscle fibers exhibited an acute degenerative change characterized by pyknosis and loss of nuclei, and swelling and fragmentation of the cell body. In addition, these necrotic muscle fibers showed a very pronounced tendency to take up calcium salts. With hematoxylin and eosin stains, the cell fragments, and sometimes whole fibers, were converted into dense, opaque, deep bluish masses as a result of the calcium deposition. The inflammatory reaction in this tissue varied within rather wide limits. Proliferation of connective tissue elements and a moderate infiltrate of mononuclear cells were the usual findings (Fig. 16). Polymorphonuclear leukocytes were extremely rare.

Valve Cusps. Localized and somewhat granulomatous appearing lesions were found in the valve cusps of 2 control animals, and in 6 of the treated group. The changes ranged from a localized swelling and edematous separation of collagen fibers in association with a dense infiltrate of lymphocytes and mononuclear cells (Fig. 9), to lesions of a more diffuse character, in which there was considerable thickening of the entire valve cusp by fibroblastic activity and collagen deposition (Fig. 7). In our material, the histopathologic change which most resembled an Aschoff nodule was found in a control animal (Fig. 8), and even here the resemblance was but superficial. There was nothing in the histologic appearance which would serve to distinguish the lesions

in the valve cusps of the treated animals from those of the control series. These changes were found for the most part in the mitral and occasionally in the aortic valve.

Valve Rings. In contrast to the myocardium and the valve cusps, lesions were seen in the valve rings of the treated animals only. These lesions, which were found in 10 animals, consisted of subacute and chronic proliferative focal inflammatory reactions. In these areas the collagenous fibers showed swelling and hyaline changes and the fibrocytes showed proliferative activity. In addition to moderate numbers of mononuclear leukocytes and lymphocytes, occasional large cells with 2 to 5 nuclei and an abundant, granular, basophilic cytoplasm were seen in these foci (Fig. 10). Clustering of nuclei in a palisade fashion about a focus of swollen hyaline collagen was seen occasionally (Fig. 11). Comparable areas of focal inflammatory change were not seen in the valve rings of any of the control animals. All of these ring lesions were found in the mitral area except one which was present in the aortic ring.

Knee Joints

Knee joints were not routinely examined during the first portion of the experiment. As a consequence the knee joints from only 14 control and 53 treated animals are reported upon here.

Difficulty in assessing the effectiveness of the treatment in causing inflammatory lesions in the joints was experienced because of the presence of tissue alterations in both control and treated animals. When present, the changes were found most frequently in folds of synovial membrane at the base of the semilunar cartilage and in the angle formed by the fusion of the joint capsule with periosteum. The maximum changes seen in the knee joint of any control animal are represented in Figure 12. The changes seen in the control animals consisted primarily in slight proliferative tendencies in the lining cells of the synovium, together with a slight increase in density and cellularity of the underlying connective tissue. An inflammatory leukocytic exudate was never encountered in the control animals, and constituted one of the chief points which served to distinguish the degenerative changes of untreated animals from the definite inflammatory response seen in the joints of 6 of the treated animals (Figs. 13, 14, and 15).

These 6 animals were the only ones of the treated group showing joint lesions which we attributed to hypersensitivity. Two of these presented deformity and limitation of movement during life, and at autopsy pink, pitted erosions of the articular cartilage were seen. All presented cellular exudates and some hypertrophic villus formation. The duration of

treatment or number of injections was not obviously related to the frequency or severity of the lesions. The tissue lesions were more marked than the most extensive changes of the control group. The lining endothelial cells were swollen and blended indistinguishably with underlying mononuclear cells two to five layers in depth in several cases. The underlying collagen fibers showed swelling and hyalinization, and the increased vascularity was more severe than any seen in the control series. Degeneration of collagen going on to necrosis of the tissue of a villus is shown in Figure 15. The inflammatory exudate consisted of lymphocytes and large and small mononuclear cells, which showed no tendency to cluster into granulomatous nodules. Proliferative activity of fibroblasts was conspicuous. In the most severe lesion taken from one of the animals which showed gross alterations, of which Figure 14 is representative, the general reaction was of a more acute, diffuse inflammatory nature. The subsynovial tissues were edematous, hyperemic, and diffusely infiltrated with inflammatory cells. The swelling resulted in definite villus formation. A few fragmented pyknotic nuclei were seen, and some polymorphonuclear leukocytes. The small muscular arteries were prominent and swollen, although no degenerative or inflammatory changes were seen in their walls.

Muscles

Samples of striated skeletal muscle from the paravertebral region, the laryngeal region, and thigh were sectioned. In the treated group 15 animals showed varying degrees of necrosis. In 10 of these, small clusters or isolated fibers were altered; in 4, numerous small clusters of affected fibers could be found in a single low-power field (Fig. 17); in one, the foci of muscle necrosis became confluent, and large areas of muscle showed acute degeneration and inflammatory changes (Figs. 18 and 19). These muscle changes were characterized by swelling of the fiber with loss of striation and cellular detail, vacuolation, and fragmentation. Perhaps the most striking feature of the histopathologic changes was the opaque bluish red appearance of altered fibers resulting from their pronounced tendency to take up calcium salts. The inflammatory reaction associated with these degenerative changes varied within rather wide limits. In some cases an infiltrate of polymorphonuclear leukocytes brought about a rapid solution of the affected fiber (Figs. 18 and 19). In the neighborhood of those fibers rendered particularly dense by calcific deposits, the inflammatory reaction was often somewhat granulomatous, with proliferation of sarcolemmal nuclei and an abundance of large, occasionally multinucleated, giant cells (Fig. 19).

In the wall of the stomach in 2 cases, and in the smooth muscle of the

small intestine in one case, foci of acute necrosis of muscle fibers were observed. The inflammatory exudate was not as pronounced as that seen in the striated muscle, nor was there any foreign body reaction with the formation of multinucleated giant cells. Allowing for the differences in tissue, it was believed that the focal necrosis of skeletal muscle, cardiac muscle, and the foci of necrosis in the wall of the gastro-intestinal tract were entirely comparable lesions.

In the control group, lesions similar to the above were not found.

Lungs

The pulmonary arteries of 5 animals in the experimental series showed an acute and subacute arteritis which was identical with that seen in the vessels of other viscera (Figs. 2 and 3). In addition, the main branches of the pulmonary arteries in 3 of the above cases showed inflammatory changes of the intimal endothelium and subendothelial tissue similar to those seen in the aorta (Fig. 6).

As well as the above changes, there was observed in 5 instances a lesion which involved vessels, but took the form of a dense accumulation of round and spindle-shaped mononuclear cells in the adventitial and subendothelial layers of the vessel wall (Figs. 20 and 21). This accumulation involved small and medium-sized arteries and veins. While small numbers of lymphocytes were included in the dense mantles of cells about these vessels, the reaction appeared proliferative rather than exudative. The layer of cells present on the inner lining of the vessel wall was occasionally of sufficient thickness to occlude the vessel lumen completely. Fibrinoid necrosis of the vessel wall was not seen in these lesions.

One rabbit received 6 massive injections of serum and died of anaphylactic shock immediately following the injection of the seventh large dose. Most of the pulmonary capillaries in this case contained a dense, somewhat refractile, eosinophilic, homogeneous substance not unlike hyaline thrombotic material. Hemorrhage into the alveolar spaces was not seen, nor were there any leukocytic collections in relation to the capillaries which appeared to be occluded. Seven other animals died in anaphylactic shock, but this change was not identified in these or any other of the experimental animals.

Lesions comparable to the above were not seen in the lungs of any of the control animals.

Discussion

From the observations of the preceding experiments, it was concluded that we had produced by one or more massive intravenous doses of horse

serum in rabbits an arteritis basically similar to the lesions of periarteritis nodosa in man. Skin tests for sensitivity to horse serum yielded an Arthus reaction in all treated animals, varying from a hyperemic edematous area 1 cm. in diameter to a sloughing necrotic lesion 6 cm. in diameter. No positive result was found in the control areas of the treated animals or in the untreated control animals. This evidence of tissue hypersensitivity indicates that the arteritis may have its pathogenesis in the reaction of hypersensitive tissues. We have observed also in the hearts and in the knee joints of these rabbits, lesions of a rather nonspecific inflammatory character but possessing some features compatible with the lesions of rheumatic carditis and rheumatoid arthritis, respectively, and which are similar to lesions which other investigators[4,5,8,9,43] claim to have produced by experimentally induced hypersensitivity to foreign protein. In addition, lesions were noted in the lungs and in smooth, cardiac, and skeletal muscle which have no precise histopathologic counterpart in man. As a group, the experimental lesions encountered in this study were more similar to the lesion-complex of human serum sickness[30-44] than they were to the lesions of any definite spontaneous disease entity of man.

The occurrence of an arteritis in 61 per cent of the 77 treated rabbits in our series confirms the findings of Klinge,[4] Vaubel,[5] Knepper and Waaler,[13] Rich and Gregory,[12] Hopps and Wissler,[43] Fox and Jones,[26] and Apitz,[45] who have described similar lesions as occurring in 30 to 85 per cent of rabbits injected with numerous foreign proteins by a number of parenteral routes. The numbers of animals used in some of these experiments[12,43] were not sufficient to yield a result of statistical significance, and the findings in control animals were not recorded in others.[43] The failure of Smith and Zeek[46] to confirm the observation of Rich and Gregory is probably not significant inasmuch as they utilized the variation of Rich and Gregory's procedure which would be least effective in producing an arteritis in a very small series of animals. A more significant negative result has been reported recently by Alston, Cheng, and Short,[47] who faithfully followed the procedure of Rich and Gregory on a large series of rabbits. This indicates the importance of factors as yet undefined in the development of these lesions. In our experiments, the series was large enough and the incidence of arterial lesions great enough to establish statistically significant results indicating a causal relationship between this method of treatment and the resultant arteritis. While a hypersensitivity response would seem to be the most likely mechanism of this relationship, it may be possible, as Ehrich[48] has recently re-emphasized, that tremendous doses of antigen as used in

our experiments may actually produce reactions in vessels as a result of a direct injury resulting in breakdown in the defenses rather than by an actual immune response.

The basic morphologic similarity between the experimentally induced arteritis and that seen in periarteritis nodosa was striking, and has already been emphasized by others.[5,12,13,43]

Slight differences between the experimental and human lesions were present. The cellular exudate was composed predominantly of large mononuclear cells. Further, in the rabbits under the conditions of this experiment, the majority of inflamed arteries were found in the heart, while the kidney and spleen were free of such involvement. In man, the distribution of the arterial lesions in the various organs is substantially different.[49] These appear to be minor quantitative rather than qualitative differences, and possibly may be explained on the basis of species differences. Thus, the similarities are sufficient to suggest that the human disease, periarteritis nodosa, may have its pathogenesis in a hypersensitivity reaction.

This is further supported by the frequent association in man of polyarteritis with other definite allergic conditions[1,12,19] and by the observation of the lesions of periarteritis nodosa in cases of drug hypersensitivity[14-16,28] and serum sickness.[31] These facts do not define the cause of periarteritis nodosa, but they do present good evidence that the pathogenetic relationship of hypersensitivity to periarteritis nodosa may involve more than one antigenic etiologic agent. This is in accord with the observations of Knepper and Waaler[13] that a variety of antigens may be utilized to induce hypersensitivity, and result in lesions that are histologically identical. For the present, however, it must be admitted that while a survey of the literature relevant to human periarteritis nodosa is productive of highly suggestive evidence, the number of reports in which a definite relationship between the action of specific antigenic agents and the production of such arterial lesions can be proved is still too small to allow of any sweeping generalizations regarding the exact nature of the relationship of hypersensitivity to the development of the usual case of spontaneous periarteritis nodosa.

While we observed cardiac lesions similar to those reported in the experiments of others,[4,5,8,9,43] our conclusions were not in agreement with these authors, who claim to have induced cardiac lesions in rabbits by means of experimentally induced hypersensitivity, which are similar to those of rheumatic fever in humans. A careful analysis of their work casts some doubt on the validity of the conclusions drawn from some of these experiments. Klinge[4] used only 5 rabbits in that portion of his

experiments in which he describes a carditis. The only convincing photograph is that of an Aschoff-like perivascular scar, while the picture of the valvular lesion shows nothing more than mucoid swelling found in many normal stock rabbits. Vaubel,[5] who listed changes in the heart muscle in 33 of 47 treated rabbits, does not present any relevant illustrations, and much of the description suggests changes now known to be found in normal untreated rabbits.[42] The incidence and precise character of cardiac lesions in the series of Hopps and Wissler [43] seem open to question, since the illustrations of their lesions included the obvious spontaneous myocarditis of rabbits described by Miller.[42] On the other hand, the pictures of valvular and Aschoff-like lesions in the myocardium in Rich and Gregory's paper [9] are admittedly convincing of the similarity of these lesions to those of rheumatic carditis. They found such lesions in 19 of 51 treated rabbits, but failed to report on the incidence of similar lesions in control rabbits living in the animal house at the same time under the same conditions. The latter consideration is important in the light of our observations on the presence of such lesions in control animals in an incidence not significantly different from that in the treated group.

Whatever the etiology and pathogenesis of some of the cardiac lesions which these authors have attributed to hypersensitivity, and which we found in both control and experimental animals, there is no question of the fundamental morphologic similarity between them and the lesions of rheumatic carditis in man. In our material, in both treated and control animals, the myocardium showed foci of spontaneous myocarditis in approximately the same incidence as that reported by Miller.[42] In the valve cusps of the treated animals, inflammatory foci of mononuclear leukocytes and, occasionally, well formed granulomata were seen, but similar lesions occurred in the control animals. The inflammatory foci encountered in the valve rings of 10 treated animals bore some resemblance to Aschoff bodies in that there was swelling and necrosis of collagen fibers, palisading of nuclei, and even the formation of giant cells in small numbers. It is true that similar lesions were not found in the valve rings of the 25 control animals. While the valve rings and valve cusps were considered separately in the tabulation of results, it is not reasonable to regard them as dissociated functional and anatomic entities when the pathogenesis of the inflammatory lesions seen there is considered. The total incidence of inflammatory lesions of the valves and valve rings is 2 in the 25 controls (8 per cent) and 16 in the 77 treated animals (20.8 per cent). While this higher incidence in the treated animals is suggestive, the difference is not actually statistically significant. Thus,

while it is apparent that the valvular cardiac lesions that we have described are in some respects similar to those of rheumatic carditis in man, we are unable to conclude that they were lesions of hypersensitivity; but it must be recognized that species differences might well result in considerable morphologic dissimilarities between the lesions in the rabbit and in man, and that a suitable variation of this experimental procedure might considerably reduce the dissimilarity and increase the frequency of such experimental lesions. The fact remains that under the conditions of these experiments our observations have failed to prove that lesions with the characteristics of rheumatic carditis can be produced experimentally in rabbits by this method. Moreover, it appears to us that no other experiments have as yet yielded unmistakable proof that such lesions may be induced on the basis of a reaction of hypersensitive tissues. The similarity of the lesions in control and experimental animals to those of rheumatic carditis, while not giving evidence as to their cause, does suggest that the rabbit is capable of reacting to injury with the production of lesions of this character.

In the lungs the rather unusual changes in vessels, characterized by dense perivascular and intimal collections of large mononuclear cells, were found in 5 of the treated animals, and were not seen in any of the control group. Pentimalli[50] described and illustrated similar lesions in the lungs of rabbits following the parenteral administration of foreign serum. While the percentage incidence of this type of lesion in our material was so small as to raise some reasonable doubt, hypersensitivity cannot be ruled out as the pathogenic agent in their production until the study of larger series is made. Another lesion, found in only one rabbit, consisted of changes involving pulmonary capillaries. In a series of 26 animals, Knepper and Waaler[13] reported the occurrence of hyaline thrombi in the pulmonary capillaries of 8 animals dying of anaphylactic shock. Gregory and Rich[10] studied the lungs of 56 rabbits which had received large intravenous injections of horse serum, and described the presence of focal capillary damage in 10 with capillary thrombi in 9. Of this group, 2 animals died in acute anaphylactic shock, 4 more died within 24 hours, and 3 lived several days after a shocking dose of horse serum. Gregory and Rich believed that these lesions were comparable to those of pneumonitis in acute rheumatic fever and the pulmonary changes of sulfonamide hypersensitivity. In our experiments, 8 animals died of the effects of anaphylactic shock, and of these, one showed capillary plugs of hyaline material not unlike thrombus material. Comparable changes were not seen in any other animals. In some of the lesions illustrated by Gregory and Rich, an early, slight leukocytic

response is seen. Our isolated case showed no such inflammatory reaction. It would seem reasonable that if this histopathologic change is to be regarded as a part of a hypersensitivity reaction, it must be with the proviso that its occurrence seems to be associated with severe anaphylactic reactions, frequently involving the death of the animal.

In addition to the massive necrosis of heart muscle which was observed in 7 animals, and necrosis of striated muscle in 15, necrotizing lesions of smooth muscle were observed in the stomach wall in 2 cases and intestinal muscle in one case. These lesions were found only in the treated animals. The leukocytic reaction in the striated muscle lesions was more pronounced than that seen in the heart and intestine, but we feel that if some allowance is made for tissue differences, the three lesions might be considered to be basically the same. The illustrations of Klinge[4] and of Vaubel,[5] who produced these lesions in small numbers of skeletal muscle fibers close to joints following multiple serum injections, are qualitatively similar, although apparently not as extensive or as severe as those seen in our material. More recently Steiner, Freund, Leichtentritt, and Maun[51] have described focal inflammatory lesions of skeletal muscle and peripheral nerves in cases of rheumatoid arthritis, but Clawson, Noble, and Lufkin[52] found similar lesions in control autopsy material. While hypersensitivity may be a factor in the development of such lesions, their lack of specificity is further indicated by the fact that they have been found in rabbits under such a wide variation of circumstances as cholesterol feeding[53] and infection and sensitivity to streptococci.[54]

Many opinions exist, some supported by experimental observations, regarding the cause of rheumatoid arthritis.[4,55-60] Our experimental findings, like Klinge's,[4] indicate a possible relationship between hypersensitivity and the development of chronic inflammatory arthritis in rabbits. While the lesions described were nonspecific and represented, for the most part, a quantitative change from abnormalities seen in the joints in control animals, it is important to note that a definite inflammaory component was added to the changes seen in the knee joints of 6 of the treated animals, and in one of these degeneration of connective tissue was present similar to the fibrinoid necrosis of arterial walls. Furthermore, 2 of the animals presented clinical and pathologic features similar to those of well advanced active rheumatoid arthritis in man. In these there were pannus formation, pink, pitted erosion of the articular cartilage, limitation of movement, distortion, and disorganization of the joint. Unfortunately, the results do not warrant an absolute conclusion concerning the relationship of the experimentally induced hypersensi-

tivity to the occurrence of the joint lesions. Turnbull's description[60] of the remarkable improvement in many cases of rheumatoid arthritis in human beings when put on diets free of foods to which they were sensitive, indicates strongly the importance of hypersensitivity in the complex etiology of rheumatoid arthritis.

The foregoing discussion brings out rather convincing evidence that an arteritis and possibly lesions of the valve rings and knee joints of rabbits can be produced by massive intravenous injections of horse serum. In view of this it is interesting that Alston *et al.*[47] failed to produce an arteritis in rabbits in an adequate experiment using identical methods of treatment. This indicates the importance of other factors in the development of such an arteritis in rabbits made hypersensitive to horse serum. There remains the question of whether the fibrinoid degeneration of these lesions is a specific manifestation of a reaction of hypersensitive tissues, and the question of the relationship of these experimental lesions to lesions of somewhat similar morphology found in a variety of diseases in man. It is pertinent to pursue these two questions somewhat further.

A marked fibrinoid necrosis of the connective tissue and related structures was a prominent feature of the majority of the well advanced arterial lesions. Whether this latter change was the result of a flooding of the damaged tissues with a material that stained selectively for fibrin or whether the eosinophilic substance represents greatly swollen or much increased degenerated ground substance, collagen, and myoplasm, could not be determined. Whatever the origin of this material, it was associated with rather widespread necrosis of tissues in the areas where it was found. There is no reason to believe that the fibrinoid necrosis of these lesions is in any way specific for the reaction of hypersensitive tissues because, as Klemperer[61,62] has pointed out, similar changes are found in the course of some acute bacterial infections, in necrotizing arteritis of experimental hypertension, inflammatory lesions due to physical and chemical irritation, areas adjacent to pancreatic fat necrosis, the base of peptic ulcers, and in some experimental traumatic lesions of skin. It seems more reasonable to believe, as Duff[63] has pointed out, that this alteration represents the final outcome of changes occurring in connective tissue as a result of a variety of injurious agents and conditions. It would be unwise, therefore, to generalize regarding hypersensitivity as the possible basis for a variety of human diseases because of morphologic similarities between the lesions of these diseases and the experimental lesions of hypersensitivity. For this reason, it seems rather premature to consider the healing arteritis of coronary arteries of Rich and Gregory's sensitized rabbits as related to the "sclerotic" type of

arteritis of rheumatic fever and disseminated lupus erythematosus.[22] Also, from our own observations such an assumption seems unwarranted because we have seen such an arteritis in the mesenteric arteries of our sensitized rabbits (Fig. 5), which is obviously the healing stage of an acute arteritis (Fig. 2), and which, if it can be compared to anything in the human, is to be compared only to the lesion of healed periarteritis nodosa. Furthermore, such caution in interpreting the relation of hypersensitivity to lesions of this character is in line with the facts recently presented by Baehr and Pollack,[64] who stressed the lack of substantial evidence for the hypersensitive nature of disseminated lupus erythematosus and scleroderma.

So far as our material was concerned, it was not clear that the lesions resembling rheumatic carditis morphologically were due to experimentally induced hypersensitivity. Rössle,[23] Friedberg and Gross,[65] and Teilum[21] have analyzed groups of cases of combined periarteritis nodosa and rheumatic fever and have critically discussed this association and the blending of one lesion into the other in the heart in these two diseases. Rich and Gregory[8] have summed up rather convincing evidence to show that in the human, many cases of rheumatic fever present manifestations of anaphylactic hypersensitivity. The clinical association of human periarteritis nodosa and rheumatic fever, and the demonstration that periarteritis nodosa in man and similar experimental lesions in rabbits can result from serum hypersensitivity, constitute rather suggestive evidence for the view that they may well result from a common or closely related pathogenetic mechanism. Therefore, in spite of the fact that it was impossible to conclude from our results that rheumatic-fever-like lesions may be induced in experimental animals by this method, and although the experimental proof of others is not convincing on this point, we believe, nonetheless, that none of the evidence precludes the possibility of a causal relationship between hypersensitivity and rheumatic fever, and it seems reasonable that further investigations by other methods of inducing hypersensitivity may well result in a more clearly defined relationship. What has been said of rheumatic fever can be said equally of rheumatoid arthritis and the relation of our experimental joint lesions to this disease. In this connection, recent support has been given to the view that antigens of differing types and differing methods of administration are associated with immune responses[66,67] of differing types and that the lesions resulting from sensitization vary with variation in the immunization procedure.[68-74]

Even if it can be demonstrated that tissue hypersensitivity plays a determining rôle in rheumatic fever and possibly in rheumatoid arthritis,

we should still bear in mind what has already been emphasized by Aschoff[7] in regard to rheumatic fever, that if such a relationship exists, it can be only a pathogenetic one and the etiology will remain unsolved. Rackemann[55] has discussed the possible diversity of the antigenic component of such a relation and the importance of many complex local and general factors, such as inherent and changing constitution, in determining when reactions will occur, what their structure will be, and in what tissues they will develop. Such modifying factors must be taken into account before we can accept intelligently the hypothesis of hypersensitivity as an explanation for the development of periarteritis nodosa, rheumatic fever, and rheumatoid arthritis.

Summary and Conclusions

A wide variety of lesions have been attributed to experimental hypersensitivity and in some instances similar experimental lesions have been produced by other means. In man many diseases associated with lesions of fibrinoid necrosis of collagen have been attributed to reactions of hypersensitive tissues. In view of the problems posed by these facts we undertook to determine the variety and frequency of lesions that would occur in a large group of animals made hypersensitive by one method. Seventy-seven rabbits were sensitized to horse serum by means of one or more intravenous doses of 10 cc. of fresh sterile horse serum per kg. of body weight. They were sacrificed approximately 10 days after the last injection, and the brain, heart, aorta, lungs, liver, spleen, pancreas, stomach, intestines, mesentery, kidneys, skeletal muscle, knee joint, bone marrow, and thymus were studied histologically.

An arteritis morphologically similar to periarteritis nodosa was found in 60 per cent of the rabbits. While there seemed no reason to doubt a causal relationship between the arteritis and the injections of horse serum, it was pointed out that the failure of Alston *et al.* to confirm these results indicates the importance of other factors in the development of these lesions.

Our observations did not confirm the conclusions of Rich and Gregory that lesions similar to rheumatic carditis can be produced in rabbits by massive intravenous doses of horse serum. It is true that inflammatory lesions of heart valves were seen in 20 per cent of the animals which received injections of horse serum, but similar lesions were found also in 8 per cent of the control rabbits, and the one most resembling a lesion of rheumatic carditis was found in a control animal. Furthermore, no Aschoff-like lesions in the myocardium were seen. Our observations indicate that if lesions in the rabbit like those of rheumatic carditis are

a result of hypersensitivity, some method other than that used in our experiments will be necessary to demonstrate such a relationship.

Of the 53 treated animals whose knee joints were examined, 6 presented inflammatory and degenerative lesions of more severe degree than were found in the control rabbits. However, the incidence was not sufficient to indicate a causal connection between these changes and the administration of horse serum.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 58

FIG. 1. Acute arteritis of a mesenteric artery of rabbit 48. This animal received 2 injections and was killed 10 days after the second. To the right the media appears normal. To the left the media shows a moderate edema and the presence of some pyknotic nuclei. There is a mononuclear exudate in the adjacent intima and media which extends to the adventitia and intima overlying normal-appearing media. Hematoxylin and eosin stain. × 200.

FIG. 2. Acute fibrinoid necrosis of a mesenteric artery of rabbit 6. This animal received 2 large injections of serum and died of the results of anaphylactic shock several hours after a third injection of 1 cc. The entire wall is involved in an acute degenerative and inflammatory process. The attenuated straightened internal elastic membrane is seen toward the left. The media in the lower part of the photograph shows marked edema. Muscle nuclei and strands of myoplasm are all that can be seen of the media in this area. In the upper field a homogeneous, deeply eosinophilic material which stains selectively for fibrin is present. Remnants of nuclei are seen floating in it. This same material is present in the intima which also shows an accumulation of mononuclear cells. In this region the internal elastic lamina is broken and the eosinophilic material of the media and intima blend. Hematoxylin and eosin stain. × 265.

FIG. 3. Acute arteritis of a medium-sized mesenteric artery of rabbit 48. This animal received 2 injections of serum and was killed 10 days after the second. The segmental character of the reaction can be well seen, with normal appearing areas alternating with damaged segments of the arterial wall. Hematoxylin and eosin stain. × 70.

FIG. 4. Preservation of the internal elastic lamina in healing arteritis of rabbit 114. The lumen containing red blood cells is in the upper left corner. Adjacent to the lumen there is cellular infiltration and proliferation. The definite outlines of the internal elastic lamina, although altered, are seen separating the intima and the media. A part of the media shows edema and fibrous proliferation. Hematoxylin and eosin stain. × 160.

FIG. 5. Chronic arteritis of a medium-sized mesenteric artery of rabbit 27. This animal received 8 injections of serum and was killed 10 days after the last injection. The endothelial cells tend to be swollen and prominent, and the subendothelial layer is markedly thickened with cellular connective tissue. The media shows slight distortion due to scarring. Lymphocytes and large mononuclear cells are plentifully sprinkled throughout the rather dense collagenous adventitia. Hematoxylin and eosin stain. × 68.

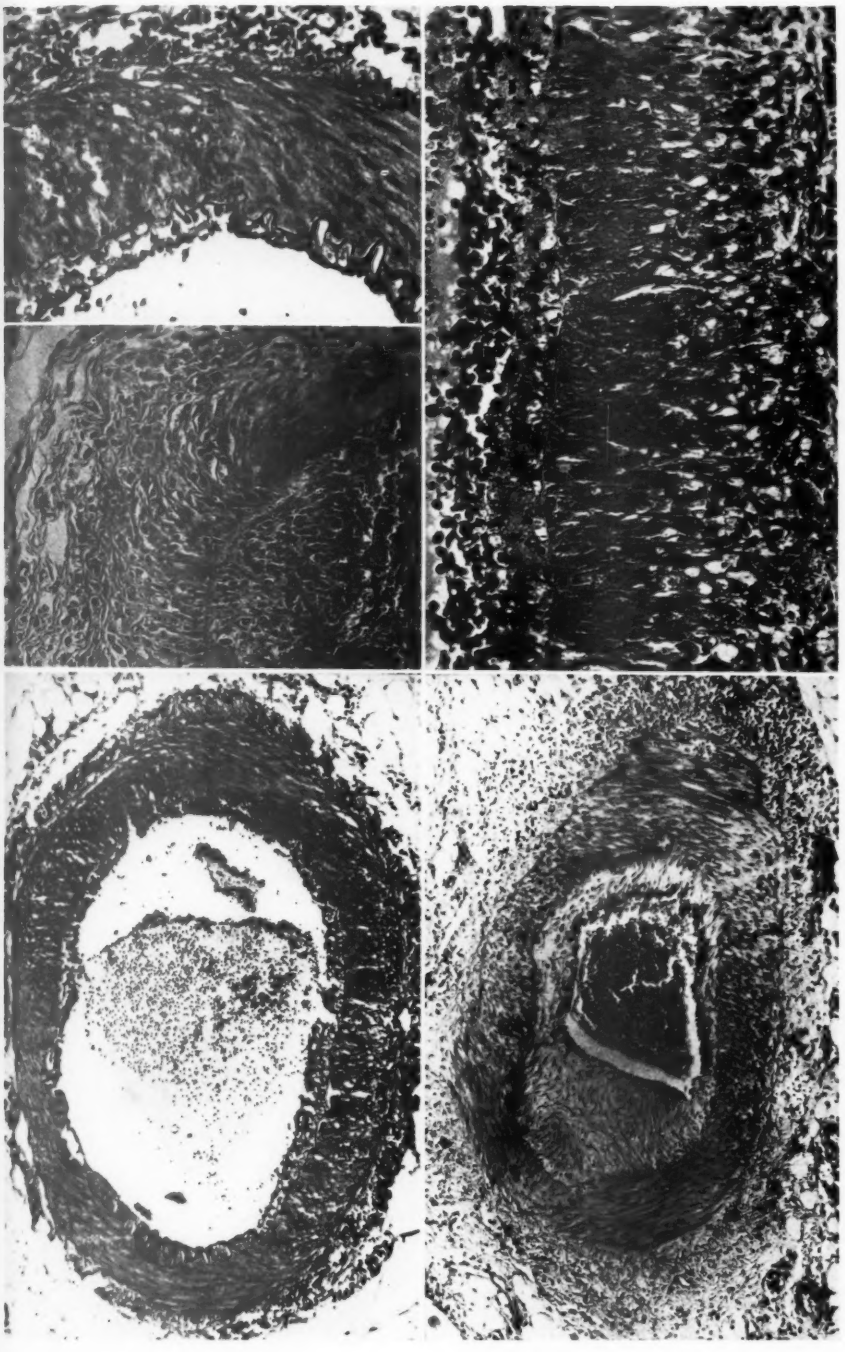

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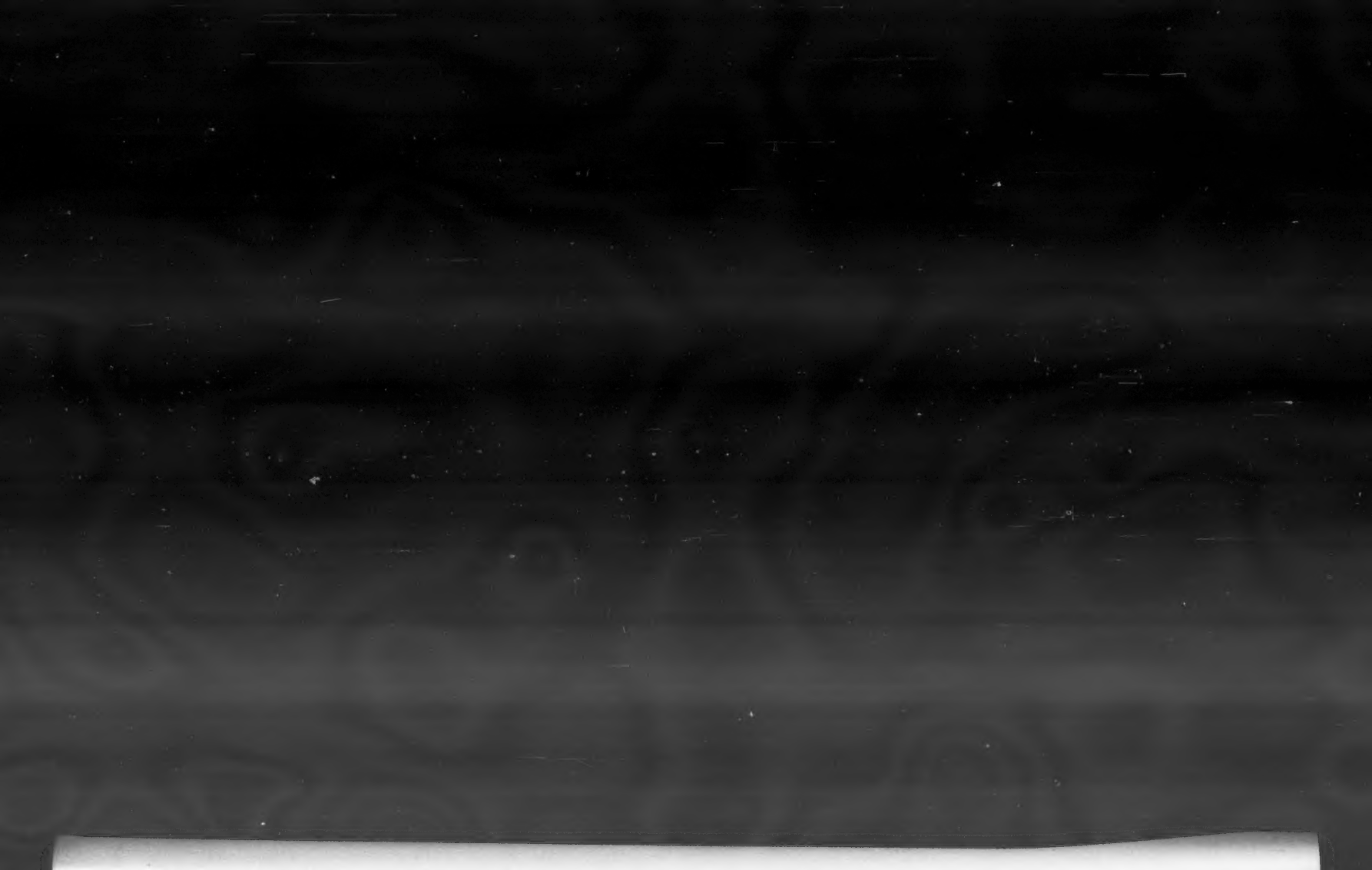
PLATE 59

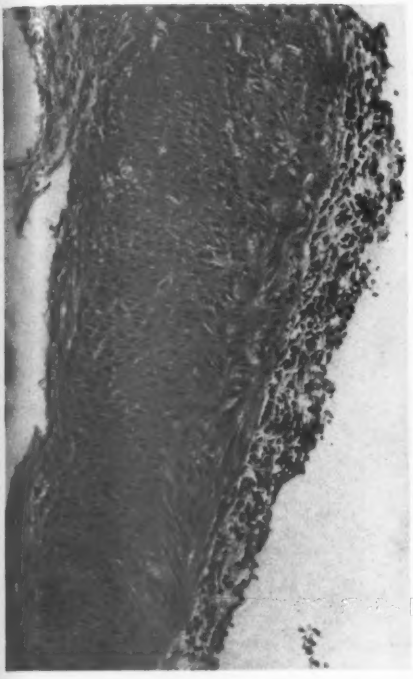
FIG. 6. Diffuse inflammatory reaction in the subendothelial tissue of the aorta of rabbit 58. This animal received 2 injections of serum and was killed 4 days after the last injection. A diffuse infiltrate of lymphocytes and mononuclear cells is seen in the subendothelial tissue. Hematoxylin and eosin stain. × 100.

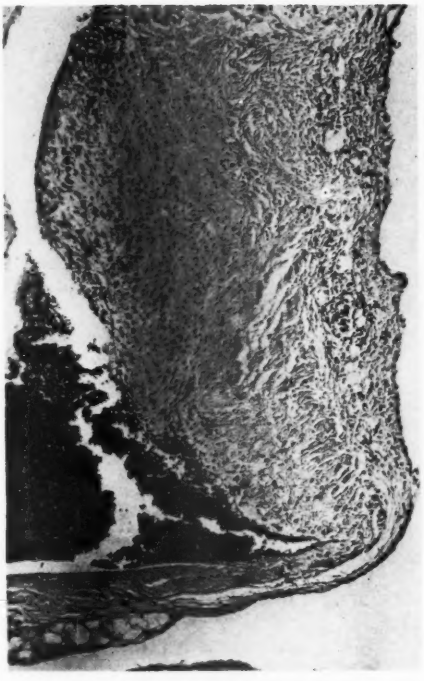
FIG. 7. Diffuse inflammatory reaction in the heart valve of rabbit 27. This rabbit received 8 injections of serum and was killed 10 days after the last injection. The free end of the valve cusp shows a marked degree of diffuse thickening, with excessive deposition of collagen, some fibroblastic activity, and collections of lymphocytes and mononuclear cells. Hematoxylin and eosin stain. × 75.

FIG. 8. Granulomatous nodule in the heart valve of control rabbit 119. An inflammatory nodule is present on the inferior surface of the valve cusp. The central portion of the nodule is pale, with edema fluid which separates the attenuated collagen fibers and contains a few inflammatory cells, chiefly lymphocytes and mononuclear cells. Swelling and proliferative changes are seen in the fibroblasts at the periphery of the lesion. This animal was not treated in any way. Hematoxylin and eosin stain. × 235.

FIG. 9. Focal inflammatory reaction in the heart valve of rabbit 40. This rabbit received 6 injections of serum and was killed 10 days after the last injection. The valve is edematous, pale, and swollen to a fusiform outline. In the central portion toward one surface is a dense collection of small round cells, the majority of which are lymphocytes. Fibrinoid necrosis of the ground substance is not present. Hematoxylin and eosin stain. × 140.







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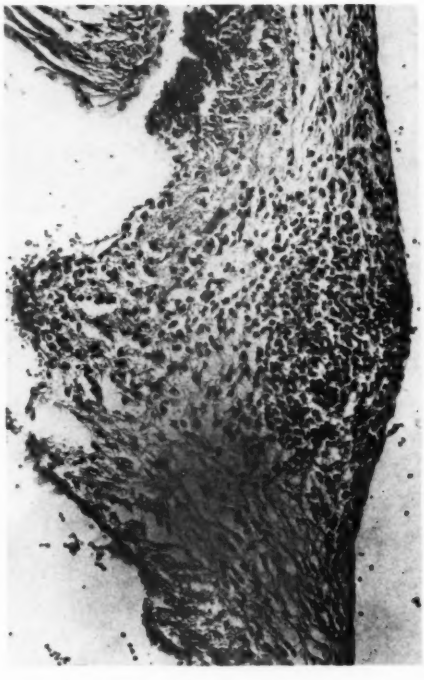

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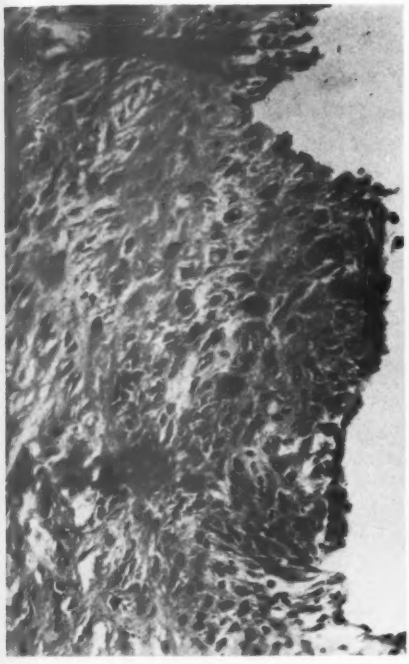
PLATE 60

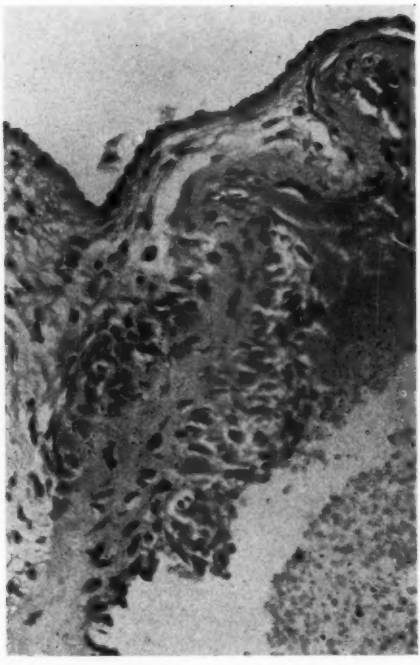
FIG. 10. Granuloma of a mitral valve ring of rabbit 72. There is edema of the connective tissue in the base of the mitral valve with fibrous thickening of the subendothelial tissue. Mononuclear and multinuclear giant cells are prominent. Hematoxylin and eosin stain. × 250.

FIG. 11. Inflammatory nodule in a valve ring of rabbit 15. This animal received 3 large injections of horse serum and died in anaphylactic shock following the third. There is a proliferative inflammatory reaction about a mass of swollen hyaline collagen in the superficial portion of the valve ring beneath the attachment of the valve cusp. The fibroblastic nuclei are swollen and hyperchromatic and show some tendency toward palisading. Hematoxylin and eosin stain. × 250.

FIG. 12. Maximum alteration of the synovial lining of a knee joint of control rabbit 13. There is some subendothelial hyalinization and a slight increase in the cells of this region. Hematoxylin and eosin stain. × 225.

FIG. 13. Moderate change in the synovial lining of a knee joint of treated rabbit 72. This is a villous formation showing edema and a mononuclear infiltration most marked toward the surface. Hematoxylin and eosin stain. × 225.





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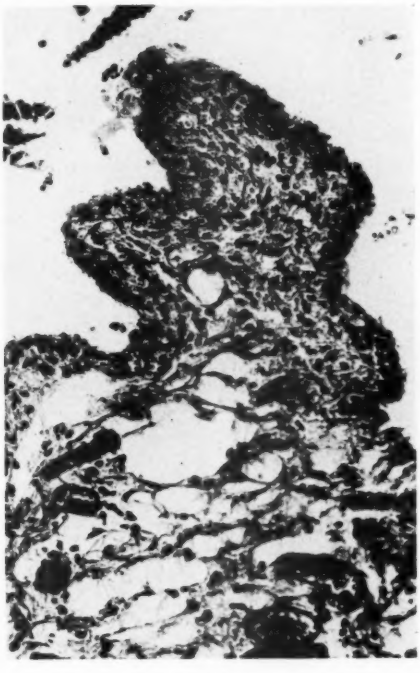

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PLATE 61

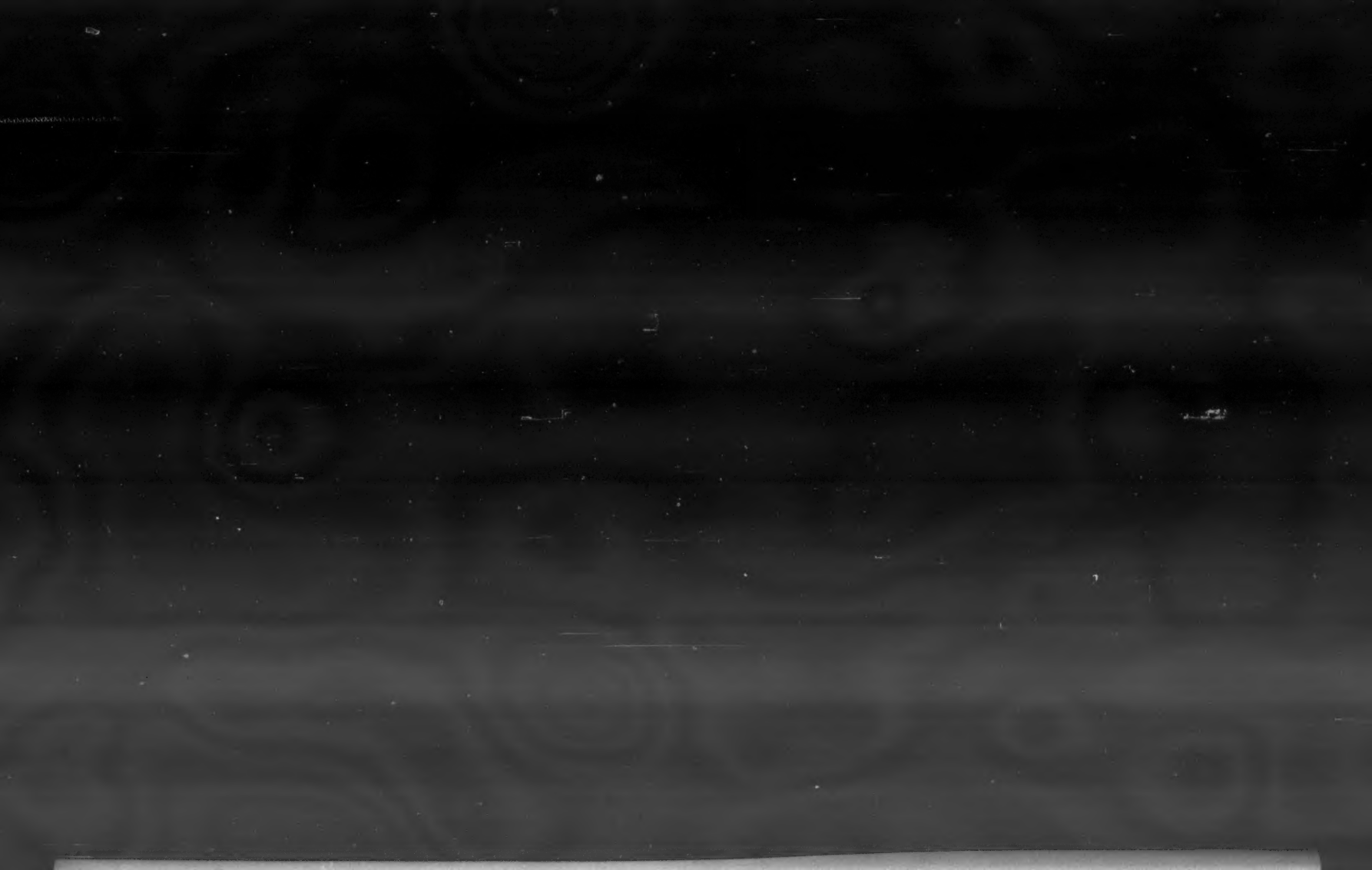
FIG. 14. Joint capsule of rabbit 27. This rabbit received 8 injections of serum and was killed 10 days after the last injection. There is marked edema of a synovial fold. There is proliferation of the synovial lining cells. The underlying tissue is edematous, hyperemic, and infiltrated with plasma cells, large mononuclear cells, and occasional polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 265.

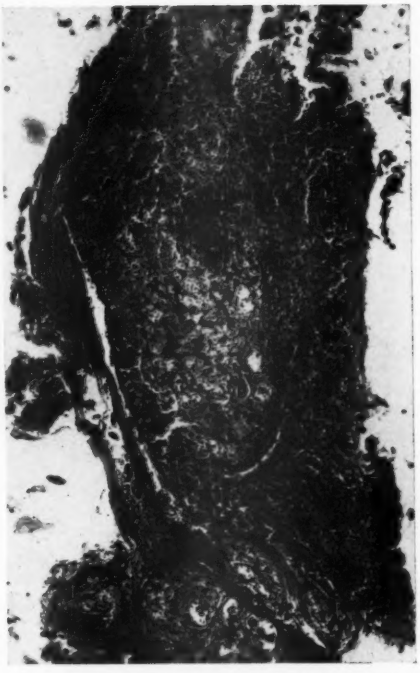
FIG. 15. Fibrinoid necrosis of a villus from a knee joint of treated rabbit 27. Some connective tissue structure and blood vessels can be made out near the center of the field. About this all structure is lost and the homogeneous material contains fragmented pyknotic nuclei. This homogeneous material was deeply eosinophilic and characteristic of the changes of fibrinoid degeneration. Hematoxylin and eosin stain. × 145.

FIG. 16. Necrosis and calcification of the heart muscle of rabbit 19. This animal received 2 injections of serum and died 7 days after the second. The darkly stained fibers are necrotic, and are stained a deep bluish color with hematoxylin and eosin. This change in staining reaction was due largely to calcium deposits, as demonstrated by the use of von Kossa's stain. Hematoxylin and eosin stain. × 210.

FIG. 17. Necrosis and calcification of the skeletal muscle of rabbit 44. This animal received 2 injections of horse serum and was killed 4 days after the second. Numerous fibers, singly and in small clusters, show varying degrees of degenerative change, with swelling, loss of striations, fragmentation, and intense basophilia due to deposits of calcium salts. Under the microscope, swelling and proliferation of the sarcolemmal nuclei may be seen. Hematoxylin and eosin stain. × 175.

FIG. 18. Necrosis, inflammation, and calcification of the skeletal muscle of rabbit 7. This animal was given 4 injections of serum and was killed 10 days after the last injection. There are many necrotic dark fibers containing calcium, and extensive areas of interstitial infiltration with mononuclear cells and a few polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 180.





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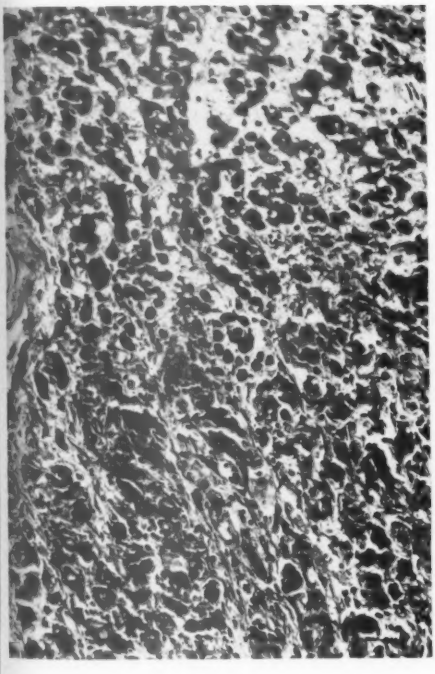

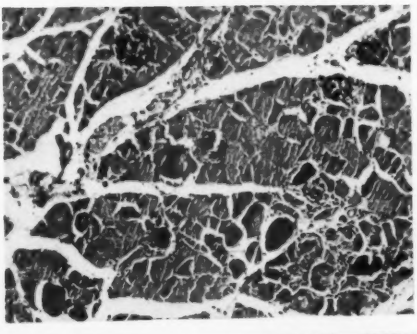

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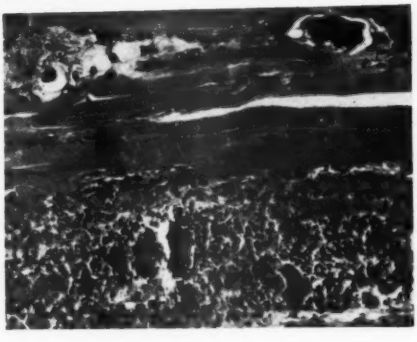

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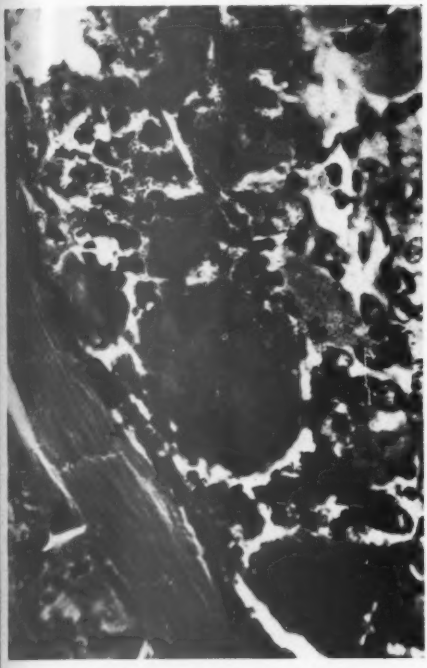
PLATE 62

FIG. 19. Higher magnification of Figure 18. The character of the cellular proliferation and infiltration can be seen. Hematoxylin and eosin stain. × 620.

FIG. 20. Paravascular granulomas of the lungs of rabbit 55. This animal received 2 injections of serum and was killed 2 days after the second injection. There are dense paravascular collections of cells. In some, the vessel walls can be seen. Hematoxylin and eosin stain. × 49.

FIG. 21. Higher magnification of Figure 18. The thin, irregular vessel wall can be seen and in a few areas appears to be completely destroyed. The character of the large mononuclear cells can be seen. These lie both within and outside the vessel wall. Hematoxylin and eosin stain. × 335.



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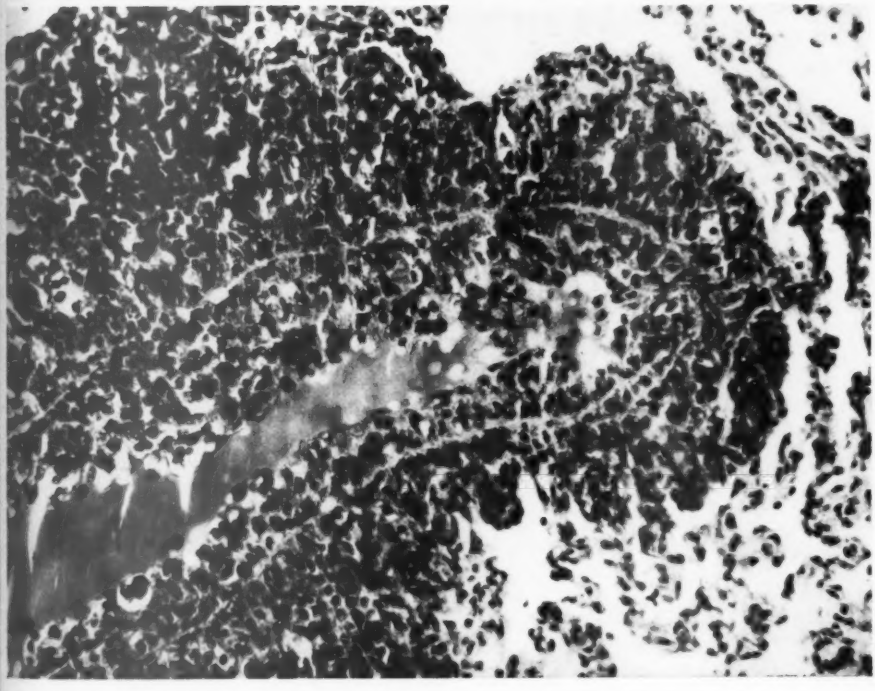

THE MORPHOLOGY OF BAUXITE-FUME PNEUMOCONIOSIS *

J. P. WYATT, M.D.,† and A. C. R. RIDDELL, M.B., D.P.H.
(From the Division of Industrial Hygiene, Department of Health, Province of Ontario, Toronto, Ont.)

A lung disease associated with the manufacture of alumina abrasives was uncovered recently through an industrial survey by Shaver and Riddell.[1,2] The manufacture of these abrasive powders, the principal ingredient being Arkansas bauxite, was greatly increased in the later years of World War II, and resulted in a new and lethal pulmonary hazard with distinct clinical, radiologic, and morphologic findings. The salient features of the morbid anatomy in 6 fatal cases are brought forward in this contribution. The industrial exposure, symptomatology, case histories, radiologic findings, and chemical assay are given briefly to complete the background of this new entity.

In the industrial process involved, an abrasive, made up of an aluminum oxide known as "corundum," is being produced. The processing is carried out in electric furnaces with the mix consisting of finely ground bauxite, iron, and coke. Carbon electrodes are lowered to the surface of the mix and fusion occurs at a temperature of 2000°C. Dense white fumes are evolved during this process, leading to contamination of the furnace rooms. All fatal cases have occurred in furnace feeders or crane operators.

The clinical syndrome common to all is shortness of breath, cyanosis, substernal discomfort, and recurrent episodes of spontaneous pneumothorax from rupture of emphysematous bullae. Percussion and auscultation of the chest reveal a variety of signs dependent upon the underlying parenchymal changes and the presence or absence of air in the pleural cavities. The radiologic evidence is so startling and different from that in other pneumoconiotic films that Shaver[1] characterized this dust disease as individual and distinct from the more common silicosis. Diffuse, irregular, lace-like, and granular shadows, greatly increased width of mediastinum, and collapse of the lung are the cardinal features of the roentgenogram.

The parenchymal fibrosis of the lungs is unlike anything previously encountered (Figs. 1 and 2).

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† Now at Department of Pathology, St. Louis University School of Medicine, St. Louis, Mo.

Report of Cases

CASE 1

H. B., a Norwegian, had spent the last 11 years of his life in the abrasive-manufacturing furnace rooms. The terminal 5 years of employment were as a crane operator above the charged steel pots. Past history, with reference to the lungs, revealed an attack of influenzal pneumonia in 1918, influenza in 1926, work in underground mines for 11 years, and intermittent rock drilling in a canal area for 12 years, but neither of these occupations entailed exposure to dusts high in silica.

Gross Examination of the Lungs

The lungs were heavy, indurated, puckered, and gray-black. The scar tissue in the interlobular septa gave a granular, pebbled appearance to the lung surface covered by thickened pleura. Pigmentation was heaviest in the regions of localized subpleural scars which extended downward into the underlying parenchyma. No pleural nodules were discernible. On section, the pulmonary tissue was pigmented. Wide regions of parenchyma had been replaced by irregular, sweeping strands and masses of dense, gray-black fibrous tissue. These masses of homogeneous scar were generally located midway between the hilum and the periphery of the lung and consisted of branching bands 2 to 4 cm. in width, with finer prolongations extending peripherally through the interlobular septa to the pleura and mesially along the walls of bronchi and blood vessels to the hilum. No discrete nodules were discovered. Between the larger areas of conglomerate fibrosis and the pleura were irregular zones of coarse emphysema. In a few areas, particularly toward the hilum, the parenchyma was gray-yellow, suggestive of pneumonic consolidation. The bronchi were of normal caliber and contained a grayish coagulum of mucoid material. The vessels showed varying degrees of perivascular scar thickening. All were patent. The tracheobronchial lymph nodes were slightly enlarged, firm, black, and without white, whorled nodules.

Microscopic Description

Microscopically, the fibrosis was diffuse, obliterated the normal architecture (Fig. 3), and was hyaline, with nothing to suggest its origin. Black pigment, obviously exogenous, was distributed in masses of charged phagocytes trapped between the heavy, swollen, collagen bands. No nodule formation as in silicosis was discovered, although the hyalinization of collagen simulated that condition to a certain extent. At the margins of larger masses the fibrous tissue extended into the septa between alveoli, greatly thickening their walls and encroaching upon the air spaces. The lung parenchyma showed marked septal swelling due

to intracellular edema and numerous young fibroblasts. There was irregular focal deposition of eosinophilic collagen. In some regions the acellular fibrotic tissue contained trapped lymphoid knots and dilated capillaries. The dense abutments of fibrous tissue were covered with stretched septal cells. Dilated emphysematous sacs were caught between the dusky hyaline strands of fibrous tissue. Pyknotic alveolar cells were trapped in the bundles of dense scar tissue. The alveolar spaces contained macrophages filled with black particles, numerous giant cells surrounding empty crystal clefts, and considerable cellular débris. Small isolated groups of alveoli revealed evidence of an acute pneumonia, but this feature was never pronounced. Dust cells were present in the foci of pneumonitis. The lung parenchyma in other regions was not remarkable except for alveolar capillary congestion.

The bronchi, surrounded by dense fibrous tissue, were filled with mucus and possessed a thickened submucosa, heavily infiltrated with lymphoid cells. The smaller bronchi caught in areas of fibrosis were distorted and filled with inspissated basophilic mucus (Fig. 4).

Arteries frequently revealed endarteritis obliterans, which involved chiefly the larger branches. Medial proliferation and hyalinization were the predominant vascular changes.

The tracheobronchial lymph nodes were remarkable chiefly for large accumulations of dust-filled phagocytes within the follicular structures. Scattered throughout the lymph nodes were minute patches of diffuse hyaline fibrosis, and a few of the lymph channels were lined with thin hyaline membranes. No silicotic nodules with redundant fibrosis were encountered.

CASE 2

J. F., a white male, 42 years old, was a furnace feeder through the years 1940 to 1944. Antecedent history was of no significance. This man's death was hastened by an acute respiratory illness, 1 year after removal from his industrial occupation.

Gross Examination of the Lungs

The surfaces of the lungs were covered by thickened pleura with remnants of adhesions. The parenchyma generally was replaced by heavily pigmented, diffuse, gray-black scar tissue without nodulation. Thick-walled bronchi and blood vessels traversed this diffuse scar tissue. In most places the fibrosis was so generalized that no crepitant lung remained. Near the periphery there were groups of dilated air sacs. Beneath the pleura in a few spots the tissue was soft, compressed, ragged, and markedly distorted, but the general appearance was that of collapsed bullous emphysema. At the bases of the lungs there was little parenchy-

mal distortion. There were numerous lobules of apparently acute pneumonia, red to yellow, in the basal gutters. The bronchial nodes were moderately enlarged, heavily pigmented, but not excessively hard. No trace of nodular fibrosis was detected.

Microscopic Description

There were numerous large areas of conglomerate fibrosis seen on microscopic examination. These masses of dense scarring were strung out in a coarse network. Heavy strands of hyaline fibrous tissue radiated throughout the parenchyma. At their margins, where the architecture of the lung was still recognizable, alveolar walls were extremely thick and composed of hyaline tissue. Deposits of black dust were heaviest in the large masses of fibrotic material. A foreign body giant cell reaction surrounded lozenge-shaped, empty clefts which were principally intra-alveolar in distribution and associated with the polynuclear pneumonic reaction. Giant and foam cells were not found within the septa.

Widespread septal hyalinization was noteworthy. This intrinsic septal change compressed, distorted, and obliterated the capillary pathways and in some regions these lesions were grouped together to form cores of ramifying, dense fibrous tissue. Large, subpleural, emphysematous areas with delimiting borders of hyaline septal tissue were frequent.

CASE 3

P. H., a white male, 33 years of age, had been a furnace feeder for 3 years. Past history was irrelevant. In the last year of employment there was a progressive increase of dyspnea, cyanosis, and sporadic incapacitating episodes of spontaneous pneumothorax. His condition rapidly deteriorated and death ensued in November, 1943.

Gross Examination of the Lungs

The lungs were similar in outline, weight, and tissue texture to those described in cases 1 and 2. They weighed 450 gm. each, and were dark with diffuse, rubbery texture. Pleural thickening was marked, with adhesions in the interlobar fissures. Innumerable emphysematous blebs were noted over the upper portions. The lungs cut with increased resistance. Dense bands of scar tissue were found, particularly in the upper portions, fanning out to the periphery. The bronchial tree and blood vessels were negative. The hilar lymph nodes were negative.

Microscopic Description

Microscopically, an important feature of the parenchyma was the distortion of the alveolar walls with an intense mononuclear and lymphocytic infiltrate along with swollen alveolar lining cells (Fig. 5). This

inflammatory septal lesion frequently was found between dense bands of old fibrous tissue which formed suspensory strands. The increased collagen was pericapillary in many of the affected septa. In some areas the collagen formed an eosinophilic cast of the septa; in other regions the septa were broad with a loose reticular and lymphoid stroma covered by swollen cuboidal alveolar cells. The lung parenchyma was distorted by giant emphysematous pockets arranged either in a fragmented cystic manner or as a latticework. These foci were lined with a dense fibrotic layer. Vessels, bronchi, and lymph nodes were negative.

CASE 4

R. L., a white male, 61 years old, had been a foreman in the alumina abrasive manufacturing plant for 15 years. For the last 4 years this man had worked as a furnace feeder. Previous to World War II, radiologic examination of his chest had revealed increased bronchovascular markings and some fine streaks of shadowing, but no specific diagnosis was made. This worker was symptom-free prior to 1941. His condition deteriorated progressively due to increased respiratory difficulty. He was totally incapacitated for the last 2 years of life and died in 1945.

Gross Examination of the Lungs

The previously fixed lung specimens were received in two portions, both resembling irregular blocks of black India rubber. The consistency was firm throughout. In the peripheral part of one region there were many dilated, ramifying bronchi measuring up to 0.6 cm. in diameter. A few subpleural, caseous, white foci were noted up to 0.1 cm. in diameter. Projecting from the medial and basal portions of the lungs were a number of emphysematous bullae measuring up to 2.0 cm. in diameter. The pleural surface was opaque, granular, and in some places had tough, grayish white and stringy tag ends of adhesions. The granularity was particularly marked over the vertebral borders. The opacity and stringy adhesions were enhanced over the anterior borders. The bronchi and blood vessels were incarcerated in this noncrepitant, gray-black, resilient tissue.

Microscopic Description

Microscopically, there were four salient features in this lung: (1) Of cardinal importance was the fine, diffuse, septal fibrosis and marked hyalinization. This septal change probably represented the earliest lesion, and the agmination of these infiltrated hyalinized septa led to massive conglomerate fibrosis (Fig. 6). Extracellular carbon drifts and birefringent particles accompanied the massive scar tissue (Fig. 7). Cubical alveolar lining cells were a feature of the secondary parenchymal changes. This reaction was most intense in association with the dense, conglom-

erate fibrosis. Occasional giant cells arose from these cubical cells. (2) There was massive fibrosis as in the other cases. This diffuse, radiating fibrosis was old, in some places hyalinized. No amyloid was encountered. (3) A banal inflammatory infiltration of the alveolar septa by mononuclear and plasma cells was noted. In many regions this reaction was within the dense, broad bands of scar tissue. In many regions the septa showed stuffed, swollen, club-shaped outlines filled with a mixed chronic inflammatory infiltrate. (4) Evidence for tuberculosis was given by clear-cut histopathologic criteria, consisting of caseous foci, with surrounding giant cell systems. In these regions there was a definite attempt at a peripheral defense fibrosis with drifts of black pigment incorporated. The morphologic features of tuberculosis were dominant and virgin in these areas and certainly not modified as in the koniophthisis of Belt.[3] The shadows of scarred pulmonary parenchyma were still present despite caseation, which indicated tuberculosis to be a late sequel. Endobronchial tuberculosis was found also. Extreme emphysema was noted in some regions and endarteritis obliterans was noted in many of the vessels. Lymphoid follicles, free of reaction, were scattered throughout the lung. The bronchial lymph nodes showed reactive hyperplasia, minimal hyaline fibrosis in the littoral spaces, and freedom from nodule formation.

CASE 5

N. C., a white male, 41 years old, had been a furnace feeder for 6 years and a crane operator in the furnace room for 4 years. Past history was irrelevant. For a period of 1½ years after December, 1945, there was progressive increase in respiratory symptoms and signs accompanied by lace-like shadows in the radiologic chest plate. This workman died 2 years after removal from industrial exposure.

Gross Examination of the Lungs

The right lung weighed 675 gm., with the pleura adherent over the upper portion. The remaining pleural surface was opaque. Along the lateral border there were a number of blebs measuring up to 1.5 cm. in diameter. The entire lung was of homogeneous rubbery consistency and cut sections revealed a diffuse, indurated, gray to rusty brown color. Neither nodules nor any gross evidence of tuberculosis could be found. The bronchi and vessels were negative. The left lung weighed 620 gm. and was similar in all respects to the right lung. The lymph nodes in the hilar regions were soft, gray-black, and somewhat enlarged.

Microscopic Description

On microscopic examination, the entire lung parenchyma was stiffened by disseminated, variegated, septal scarring. Many of the alveolar walls

contained young fibroblasts and capillary buds, or showed spotty hyalinization. In other regions fragmented, eosinophilic, collagenous material filled the alveolar walls. Overlying septal cells were cubical in areas and some alveolar lining cells were charged with coal-black refractile particles. In a few areas piling up of septal cells was noted; frequently buds were formed, filling the distorted alveolar spaces. The cuboidal septal cells often rested upon dusky fibrovascular buds. Retained secretion was frequently surrounded by cuboidal cells and occasionally by foreign body giant cells.

Reticulum, demonstrated by Laidlaw's method, was pericapillary in distribution in the least thickened of the alveolar septa. In regions of marked collagenous sclerosis there was still persistence of the alveolar pattern.

Thickening of the bronchial submucosa and a lymphocytic-eosinophilic inflammatory infiltrate were frequent findings. The vascular channels showed thickening of the outer media in the larger branches; the smaller showed endarteritis. The lymph nodes showed a preserved architecture, and no indications of silicosis were found.

CASE 6

R. M., a white male, 51 years old, had worked on the hot-change process for 4 years and then as a laborer in a winery for 1 year. Past history was otherwise noncontributory. Radiologic examination prior to his work as a laborer showed diffuse pulmonary shadows and bullous emphysema. One year later, a sudden pneumothorax developed with acute dyspnea and cyanosis, terminating in death.

Gross Examination of the Lungs

Microscopically, the left lung weighed 325 gm. and was greatly shrunken and compressed by a rough pleura, 0.4 cm. thick, which was plastered over the lower portions of the gray-black, indurated parenchyma. The cardinal feature was the presence of giant bullae around the periphery of the lung and emphysema in the upper portions. Some of these bullae reached a diameter of 4.0 to 5.0 cm. The lower part of one lung was firm and rubbery. The cut section revealed a uniform collapse of the parenchyma and a fish-net appearance in some regions. A few of the bronchi in the lower lobe were dilated.

The right lung weighed 375 gm. and was compressed throughout. It showed similar pronounced bullous formation. Cross section throughout the parenchyma revealed gray-black scarring with a rough, granular texture. The interlobar fissures were obliterated and replaced by a thickened scarred septum. Rough, gray-black, granular atrophy of the lung parenchyma was particularly prominent in the upper portions. Bronchi, vessels, and lymph nodes were negative.

Microscopic Description

Microscopically, the lung architecture in many regions was completely altered by diffuse interstitial fibrosis of a relatively mature type. The normal delicate filigree pattern was erased and the septa were thickened variably with fibroblasts and dense collagen. The septal fibrosis merged into irregular bands of old vascularized sclerotic tissue along the larger vessels and bronchi. This distribution was well demonstrated by the use of van Gieson's and Mallory's connective tissue stains. In many regions the interstitial fibrosis was sharply delineated and assumed many bizarre configurations like Chinese lettering. Smaller vascular channels showed thickening of their walls by collagenized tissue. Septal walls were broadened, stiffened, blunt-ended, and frequently were covered with hyperplastic cells. The septal lesions showed pronounced fibroblastic proliferation mingled with mononuclear cell infiltrate, and the broad, irregular bands of scar tissue were considered to be mats of such septal fibrotic lesions (Fig. 8). In the intertwined, spreading bands of old scar tissue and in the alveoli there were irregular deposits of black dust. Lymphoid follicles in the regions of interstitial fibrosis were intact. Reticulum, revealed by silver impregnation, lacked regular density and was arranged in a haphazard manner. In regions where septal fibrosis was not profound, alternating collapse and emphysematous pockets were found. Marginal emphysema was well advanced. The bronchioles within the scar reaction were dilated. A few showed squamous metaplastic changes and their lumina contained excessive secretion. Vascular channels showed thickening of their walls, narrowing of their lumina, and collagenous sclerosis outside the media of the vessels. The lymph nodes showed hyperplasia of the sinus reticulum and minimal amounts of fibrotic scarring of lymph sinusoids.

Chemical Investigation

The chemical approach to bauxite-fume pneumoconiosis offers a means of seeking the specific dust responsible for this unique pulmonary fibrosis. Investigation by chemical analysis is continuing under Dr. C. M. Jephcott, of the Division of Industrial Health, Ontario Department of Health. A few facts culled from the chemical analyses are presented in Tables I and II to offer a linkage with the described pathologic features.

Spectrographic determinations revealed that the majority of the fume particles are not greater than 0.5 μ in diameter. Analysis by roentgen diffraction shows that the fumes consist mainly of amorphous material.

From Tables I and II it is evident that amorphous silica and alumina represent the two prominent constituents. Further work has shown that

spectrographic analyses of the ashed lung residue from the fatal cases give results similar to those of the furnace fumes, thus offering a close chemical correlation between cause and effect.

TABLE I
Analysis of Furnace Fumes

Chemical	Range
Silica	29.0%—44.0%
Alumina	41.0%—62.0%

TABLE II
Chemical Analysis of Lungs

Case	Silica in ash	Alumina in ash
1	30.5%	30.4%
2	24.8%	40.5%
3	25.3%	Not examined
4	21.2%	28.9%
5	28.6%	40.2%
6	31.0%	25.7%

COMMENT

It is important to recapitulate the lung changes encountered in this entity. The specific pathologic findings were confined to the lungs. The outstanding features of the lungs from the gross examination were the relatively normal size and the gun-metal color. On palpation, the diffuse, widespread induration was of note. A fine fish-net pattern was present in some regions; elsewhere, radiating bands or masses of rubbery black tissue were more evident. Of further importance was the absence of shotty or confluent nodulation. Only in case 4 were caseous foci of active tuberculosis encountered and these had not been altered by the diffuse scarring. The diffuse fibrosis in these lungs was probably responsible for the spreading out of the anthracotic pigment and accounts for their gray-blue color. The frequency and size of emphysematous vesicles were noteworthy. The bullae were not only found incarcerated within the fibrosed parenchyma but were especially prominent in the subpleural regions and frequently reached giant size. Tags of adhesions and pleural thickening were common, particularly over the upper lobes, but pleural thickening was frequently encountered wherever the lungs had been collapsed by repeated spontaneous pneumothorax.

The hilar and tracheobronchial glands were not enlarged or hard, bore the usual amount of anthracotic pigmentation, and were completely free of nodulation.

Histopathologic examination showed a constant pattern of diffuse fibrosis. It is our belief that the initial lesion was intracellular septal edema with early fibroblastic proliferation. The next salient feature was infiltration of inflammatory cells, principally lymphocytes and mononuclear cells within the thickened alveolar walls. The fibroblastic proliferation was succeeded by collagen deposition. The irregular "starched" trabeculae made up of hyalinized alveolar walls were seen to best advantage in the material from case 5. At this stage of the morphogenetic

process, fibrosing septal walls might remain distinct, giving the stiffened reticular pattern, or these alveolar walls might be matted together and produce wide bands of scar. The fibrous scar was eventually hyalinized, either focally or diffusely. Doubly refractile, particulate matter was frequently demonstrated by polaroid examination. Distribution of these particles was irregular and haphazard in these lungs. The sites of their deposition and the particle size and shape were not of a decisive nature.

Anthracotic pigment was present in varying amounts and in a variable pattern. Its usual distribution was often disturbed and in many regions the carbon pigment was incarcerated in the dense bands of scar tissue. Within the diffuse scar tissue, bronchiolectasia and dilated alveoli filled with basophilic, trapped mucus frequently were noted. Septal cells in these sites were cuboidal, this transformation being particularly frequent where the alveoli were incarcerated in bands of collagen. Squamous metaplasia at the bronchiolar level was uncommon and not present to the same degree as the occurrence of cuboidal cells. These cellular changes are not specific, being frequently noted in diverse chronic pulmonary conditions such as those resulting from bronchial obstruction, or from cadmium smoke poisoning.

Obliterative endarteritis was frequent and reached the greatest intensity in the regions of diffuse fibrosis. Collagenous sclerosis was particularly marked in the outer portions of the vessels. There was no invasion of the walls by pigment-laden macrophages.

Confluent bronchopneumonia, tuberculosis, and lipidosis were nonspecific features found in individual cases. Acute infections are frequent but play no part in the progression of this disease. Foreign-body giant cells surrounding lozenge-shaped clefts were prominent in case 2 in the regions of pneumonic reaction. Tuberculosis in case 4 presented no special features. This pneumoconiotic background may offer a nidus for tubercle bacilli but the resulting reaction is not modified as in some other pneumoconioses, for example, siderosilicosis or the coal miner's lung. Alveolar bleb formation and emphysematous vesicles were prominent. This feature is apparently encountered relatively early in this disorder, as revealed by case 6. In the walls of some of the emphysematous blebs dense hyalinization was present. The fibrosis was pre-eminently in an interalveolar and interlobular pattern. Abundant septal scar tissue and lack of nodules are the characteristic parenchymal features in "alumina dust" fibrosis of the lung.

This tissue reaction bore no resemblance to any previously described process such as the acute interstitial fibrosis of Hamman and Rich.[4] The arrangement of the scar tissue is not that of carnifying pneumonitis of

infectious origin. There is no evidence of any specific infective granulomata or of sarcoid. Infarction and bronchial stenosis, either of neoplastic or inflammatory origin, could be eliminated in the consideration of these cases. In case 1, with a history of influenza, scars of an influenzal nature would not have persisted for the period of 25 years which elapsed before symptoms developed. No granulomatous reaction, such as that to beryllium, was present nor were asbestos bodies found; septal fibrosis is not encountered in these two lung disorders. None of the other dust diseases such as baritosis, bagassosis, or byssinosis need be considered because of the nature of the history.

All regional lymph nodes, both hilar and tracheobronchial, were free of specific inflammatory stigmata. There was neither diffuse nor concentric nodular fibrous tissue or hyaline material obliterating the lymph node architecture.

Due to the clear-cut industrial exposure and to the unique type of pulmonary fibrosis, it is accepted that this disorder occurring in workers in alumina abrasives manufacture is a dust-caused disease. The excessive, diffuse fibrosis throughout the lung tissue favors a "chemical dust" as the cause. The high silica content in the furnace fumes and in the lungs originally forced consideration of silica as the specific incitant, but this was abandoned for several reasons. Two principal objections are the existence of the silica in an amorphous form and the pattern of the pulmonary fibrosis. Only case 1 had a history of hard rock drilling, and there is no pathologic evidence of previous intense scarring from dust diseases. On morphologic grounds it is extremely difficult to believe that very finely divided vitreous silica or alumina could alter a pre-existent nodular fibrosis from rock-dust inhalation to a diffuse fibrosis.

The lungs in this pneumoconiosis lack the size, the nodulation, the configuration, and granite-like character of the silicotic lung. None of the indelible hallmarks of the silicotic reaction are present in any of these lungs. The lack of nodulation may be regarded as evidence against koniophages transmitting the inhaled particles of amorphous silica. The effect of this dust is primarily upon the septa, whereas in silicosis the original insult is borne by the lymphatics and lymphoid collections of the lung parenchyma. In this disease, the lymphoid collections are intact. Even in acute silicosis,[5,6] or in the burning of silica-bearing rock at Gauley Bridge,[20] the histopathologic reaction in the lung is reminiscent of the silicotic pattern.

The polymorphism of silicotic fibrosis, due to diffuse fibrotic plaques, has recently been stressed by Costero.[7] The silicotic nodule in his cases assumed a diffuse pattern produced either by an organizing pneumonitis

or transformation of atelectatic zones into fibrous tissue. In the pneumoconiosis under discussion, neither of these features is encountered and the fibrosis in these cases is an autochthonous process.

It has been shown chemically that these lungs contain large quantities of amorphous alumina and silica. It is necessary to consider the part played by these two substances in the genesis of the fibrotic process. That silica and certain silicates are capable of producing nodular pulmonary fibrosis is well known. Gardner[2] has shown that extremely finely divided silica does not cause a diffuse fibrosis in the lungs of animals and that in most cases the silica is eliminated from the tissues too promptly to produce any lasting harm. Extremely fine silica is inhaled in an amorphous vitreous form from the "dense white fumes," but no human disease has as yet been traced to such finely divided silica. If this is a form of silicosis, the problem still remains to explain why the anatomic form of the reaction is diffuse, not nodular. It may be postulated that another dust or combination of dusts can be held responsible for the causation of this disease.

The admixture of dusts, such as hematite[8] or anthracotic pigment,[3,19] may modify a frank silicotic process but never completely erases it. King[9] recently has demonstrated that an antidotal mixture of aluminum hydroxide with quartz lowered solubility but did not prevent experimental silicosis. Hence if the aluminum dust combined with the amorphous silica is responsible for the production of this tissue pattern, it is evoking a fundamental biologic reaction of a previously unheralded type.

In Germany[10-16] cases have recently appeared when there was a greatly accelerated production of explosives in which only alumina dust was utilized. Apparently a clinical, radiologic, and morphologic disease was produced, similar to bauxite-fume pneumoconiosis. The German experiences with "aluminum dusty lung" have been referred to by Perry[17] in a recent report. A personal communication to one of us (A.C.R.R.) indicates the existence of a similar condition in Sweden.[18]

It is suggested, therefore, that the mechanism is that of an amorphous dust evoking a rapid, sclerosing process within the pulmonary septa and interfering with the koniophage transmission mechanism usually responsible for dust elimination. The end-result is a diffuse interstitial fibrosis with absence of nodule formation. We favor considering the amorphous alumina dust in intense concentration as the dominant etiologic agent, but the final answer as to whether these unusual morphologic lesions owe their development to the combined effect of amorphous silica and alumina in the fumes or to the alumina dust alone will have to be obtained from experimental, chemical, and pathologic studies now in progress.

Years ago, Cummins, quoted by Belt,[3] stated that the "lung was an oc-

cupational log book—it retains a qualitative and an indelible record of the mineral particles breathed during life and after death constitutes a sort of palimpsest of the industrial history." With the emphasis on industrialization and the widespread use of diverse minerals, this statement has even greater significance. With new industries and accelerated manufacturing processes, new lung hazards will be followed by distinctive lung lesions.

It is our opinion, on the basis of the character of the pulmonary fibrosis and chemical analysis, that this disorder represents a distinctive pneumoconiosis.

Conclusions

Pulmonary fibrosis has been found in 6 autopsies on workers in the alumina abrasive industry. The clinical course was one of short industrial exposure and rapid development of disease. The highly characteristic lung changes are those of diffuse non-nodular interstitial fibrosis frequently accompanied by emphysematous bullae. The exact cause has not yet been uncovered, but the hypothesis is that an intense exposure to amorphous aluminum dust may play a dominant rôle in this bizarre fibrosis.

Grateful acknowledgment is made to Dr. W. L. Donohue for his help with the photomicrographs.

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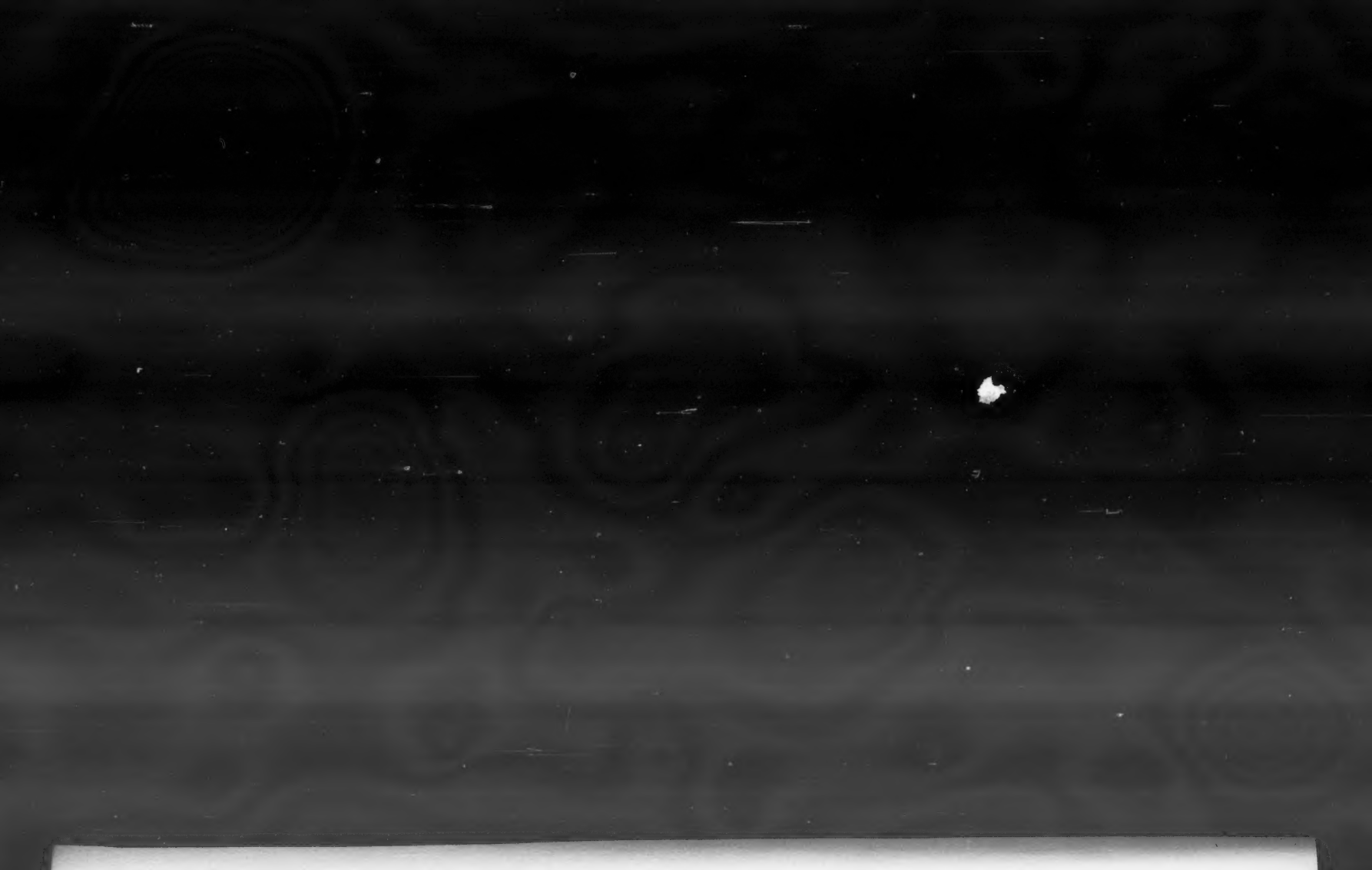
DESCRIPTION OF PLATES

PLATE 63

FIG. 1. Case 6. Collapsed, shrunken lung with widespread fine scarring. Large marginal bullae are seen. There is no evidence of nodulation.



A

V

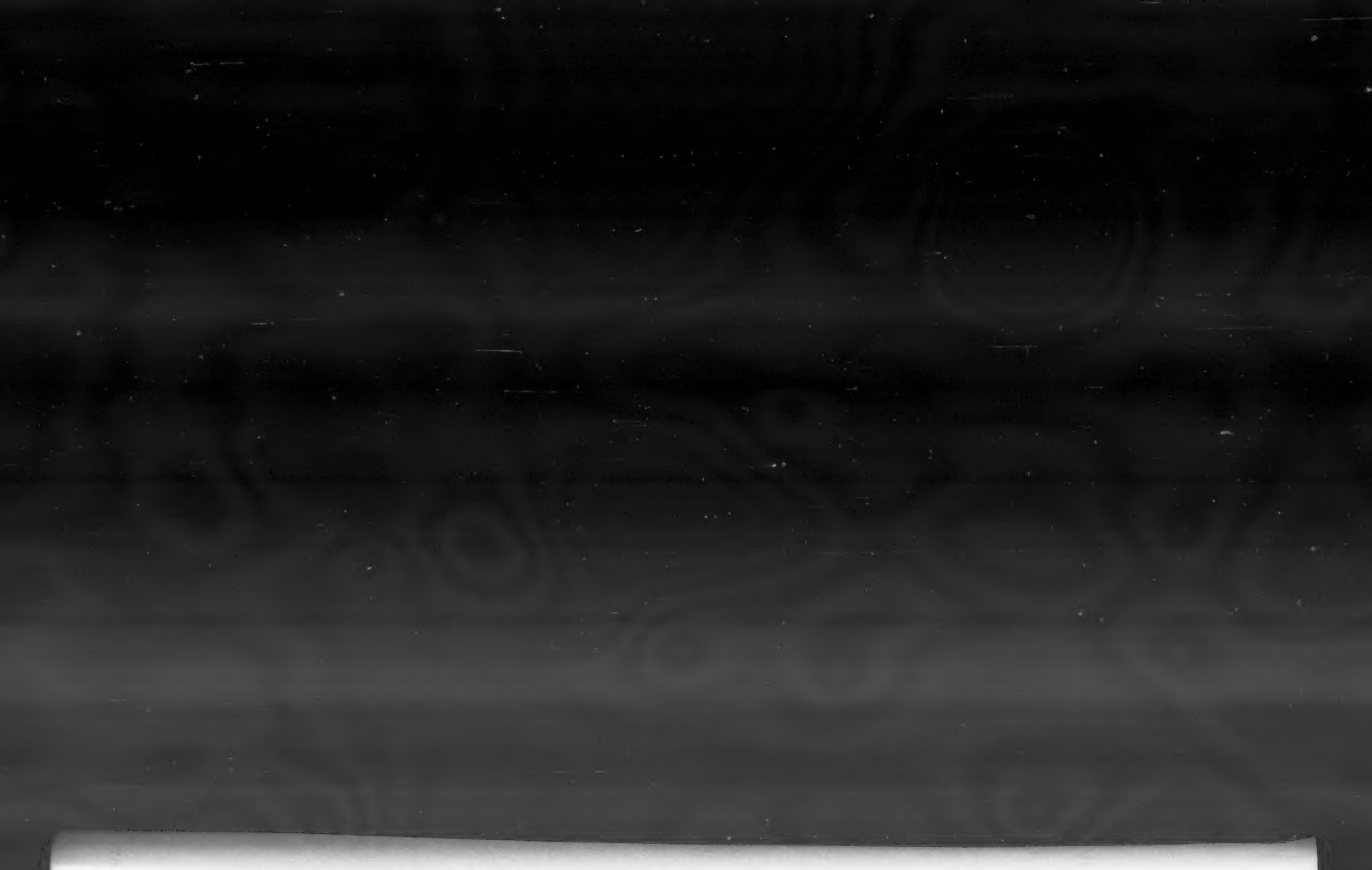
Wyatt and Riddell Bauxite-Fume Pneumoconiosis

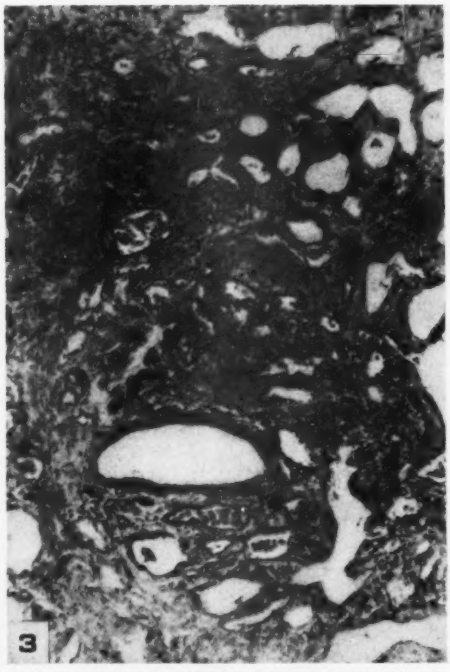
PLATE 64

FIG. 2. Lung from case 4, showing a diffuse, black, rubbery parenchyma with trapped dilated bronchial spaces. There are no nodules in the parenchyma or lymph node. × 2.

FIG. 3. Case 1. Complete obliteration of the lung pattern by scar tissue and dilated air sacs. Scar tissue is accompanied by lymphocytes. × 30.

FIG. 4. Case 1. Distorted bronchial sacs filled with trapped mucus incarcerated by non-nodular fibrous tissue. × 200.





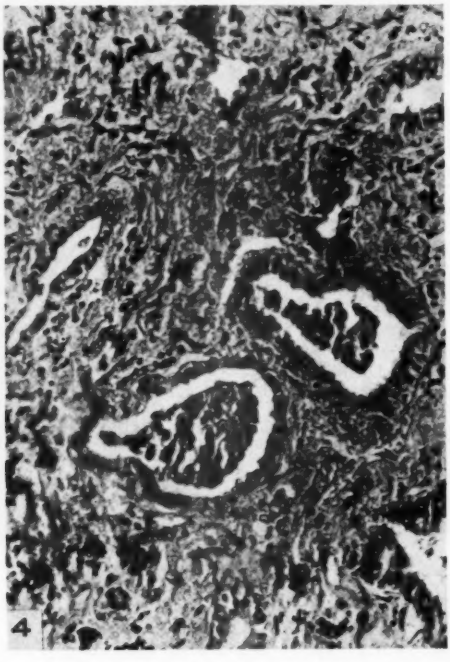


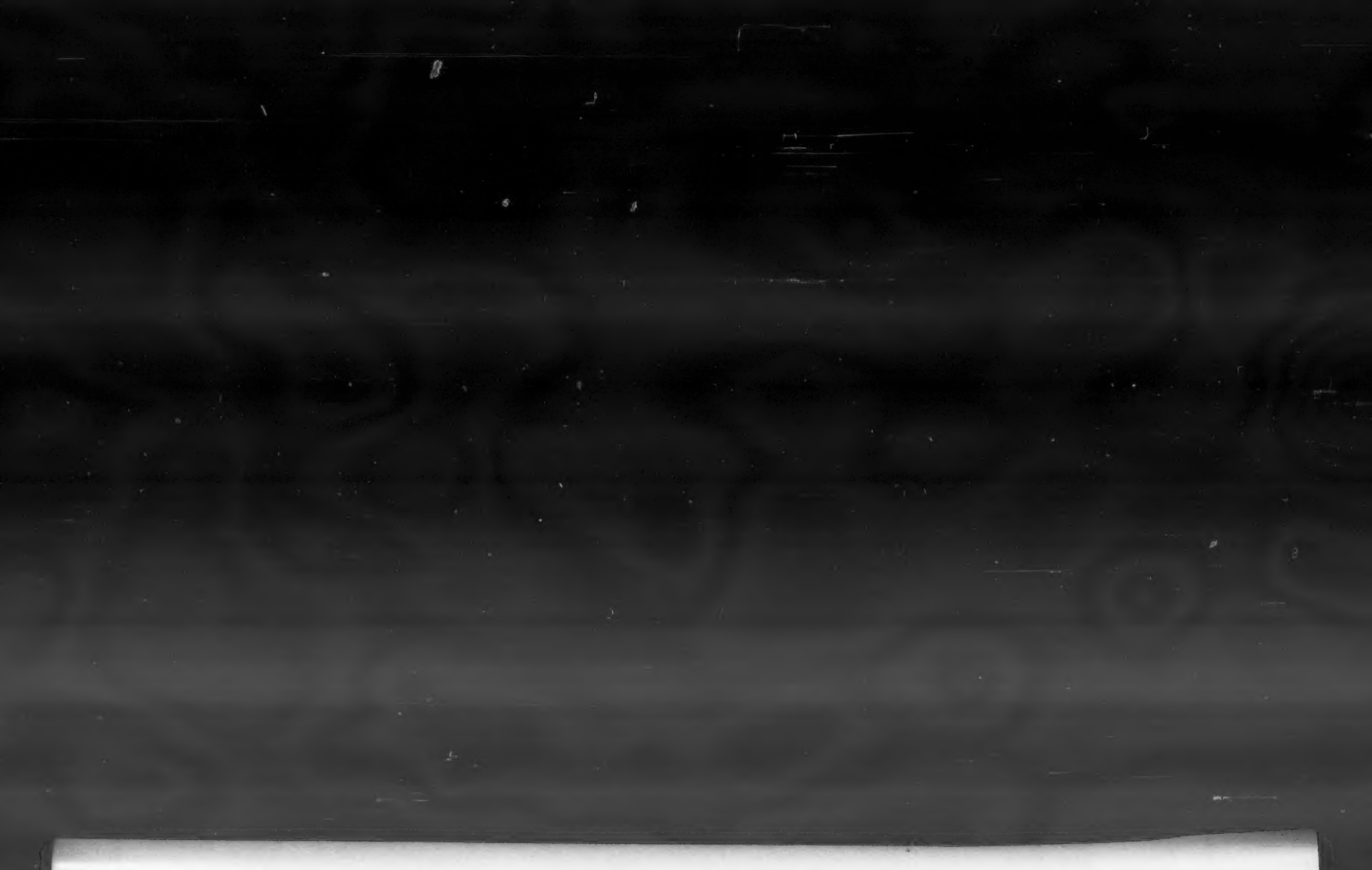
PLATE 65

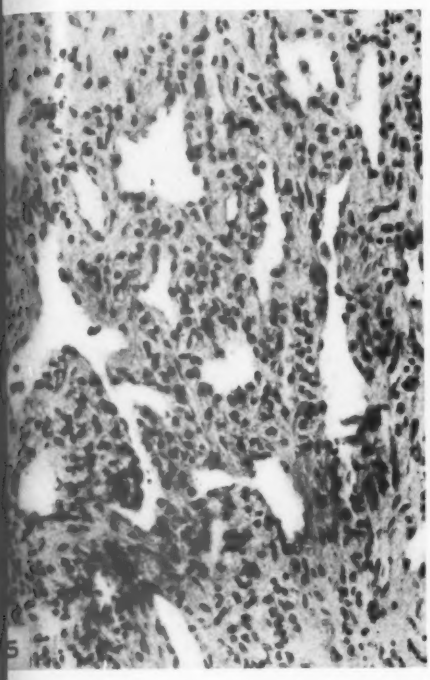
FIG. 5. Case 3. Alveolar walls are swollen and thickened by mononuclear cells and fibroblastic nuclei. Obliteration of the capillary pathway may be noted. × 450.

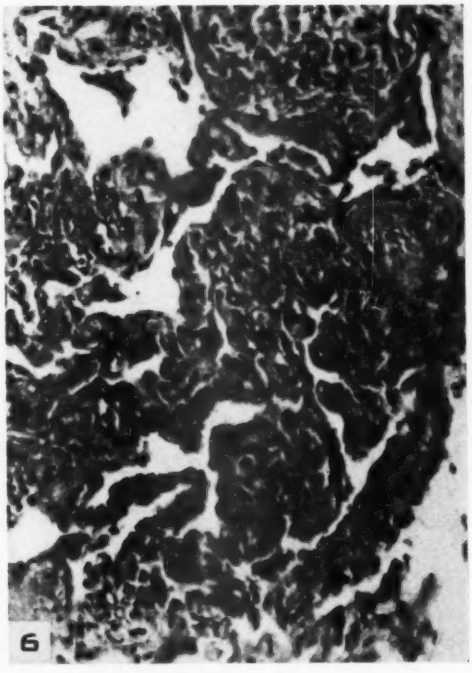
FIG. 6. Case 6. Matting together of swollen, partially collagenized, distorted septal walls. × 450.

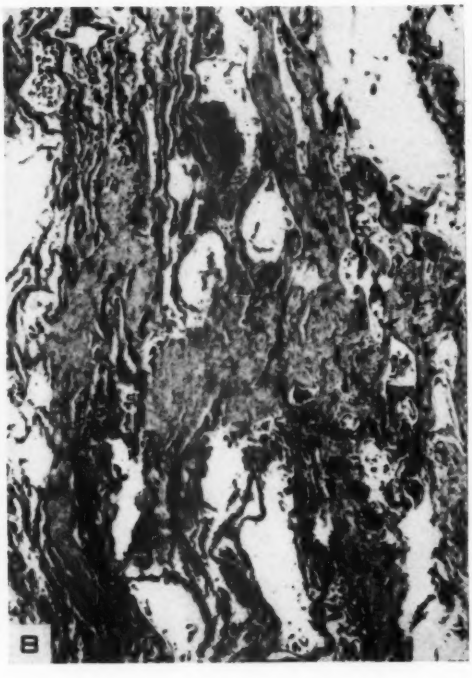
FIG. 7. Case 4. Irregular collagenization and hyalinization of septa. No nodular configuration is noted. × 200.

FIG. 8. Case 6. Dense bands and streaks of old scar tissue surrounding emphysematous pockets. × 120.









HIBERNOMA, A SPECIAL FATTY TUMOR
Report of a Case *

Osborne A. Brines, M.D., and M. Harvey Johnson, M.D.
(From the Department of Pathology, Wayne University and Detroit Receiving Hospital, Detroit, Mich.)

The thought that fat is a variable tissue composed of cells of distinct histogenesis capable of special function has been expressed by several investigators for nearly a century. In 1853 Toldt[1] stated that adipose tissue in mammals constitutes a specific organ, distinct from connective tissue. The controversy which followed this expressed concept has resolved itself into the reasonable viewpoint that at least some fat exists during primitive development as pre-adipose tissue, some of which remains in multiglobular form. Kölliker,[2] in 1856, proposed the term "primitive fat organ" which Bell[3] accepted as being the equivalent of pre-adipose tissue. Presumably some multilocular fat (brown fat) is a temporary or transitory phase of ordinary adipose tissue, while some persists as a definite permanent structure. The latter has been called "glandular" fat and is pertinent to the subject here presented (Toldt,[4] 1870; Hammar,[5] 1895). This subject was well reviewed, summarized, and presented by Shattock[6] in 1908 and by Wells[7] in 1940.

The presence of gland-like structures composed of multiloculated fat cells in hibernating animals and some nonhibernating rodents was first discovered by Velsch[8] in 1670, and since then has been studied by several workers (Cramer,[9] Hatai,[10] Vignes,[11] Inglis[12]). Various names have been employed for these structures, including adipose gland (Rudolphi,[13] 1830), brown adipose tissue (Hammar, 1895[5]), oil gland (Jones, 1852[14]), lipoid or cholesterin gland (Cramer, 1920[9]), interscapular gland (Hatai, 1902[10]), organ of hibernation or hibernal fat (Vignes, 1913[11]), and hibernating gland (Barkow, 1846[15]). The word "hibernating" has been objected to on the grounds that it is not present in all hibernating animals. Auerbach,[16] in 1902, reported his study of this organ in 26 different species; altogether, 55 species have been studied by over 50 investigators.[17] The relationship of this variety of adipose tissue to the hibernating gland of the woodchuck has been studied by Rasmussen,[17] whose presentation in 1923 brought the knowledge of this structure up to date and gave credit to the contributions of earlier workers (Text-Figs. 1 to 3). That the hibernating gland develops in relationship to the thymus, a view held by some earlier workers (Velsch, 1670[8]);

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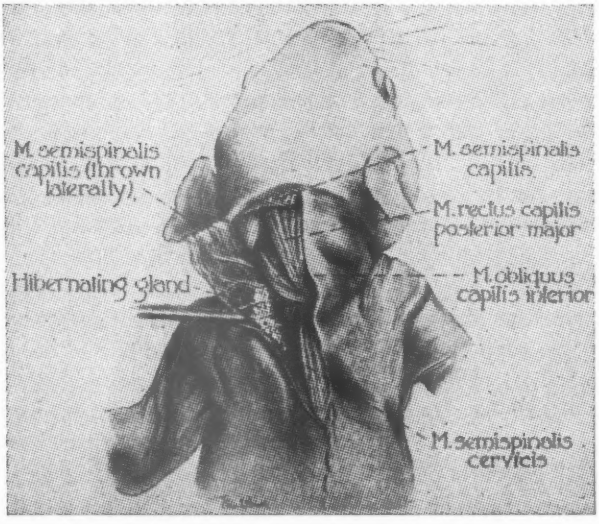
Meckel, 1806[18]), has not been mentioned by later observers. The actual transformation of ordinary fat to brown fat in hibernating animals during lethargy probably does not occur.

The hibernating gland has been described as a paired, light brown, lobulated, richly vascular mass, somewhat resembling salivary gland or pancreas, composed of cells measuring from 25 to 50 μ in diameter which are coarsely granular or multiloculated and fat-containing. The cells are about half the diameter of those of ordinary adipose tissue. The amount

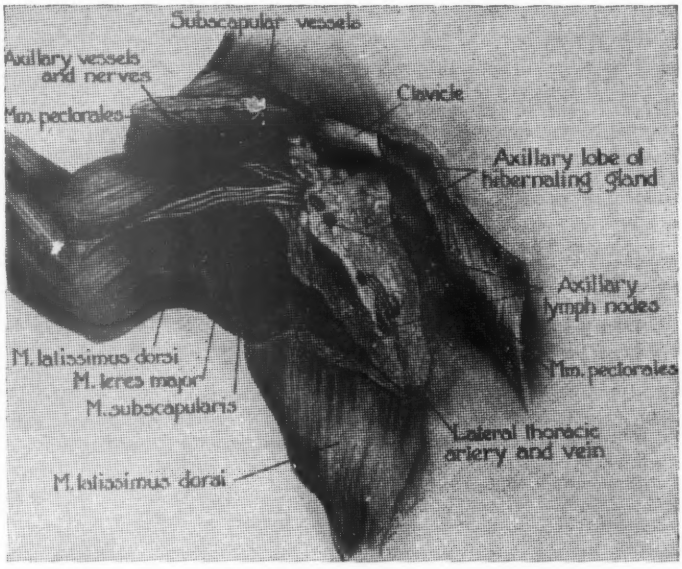


Text-Figure 1. Cervical portion of hibernating gland of adult woodchuck. (Reproduced by permission from *The Journal of Morphology*, 1923, 38, 147–205, as were also Text-Figures 2 and 3.)

of extractable fat in the hibernating gland is considerably less than that of ordinary adipose tissue (Carlier and Evans,[19] Shattock[6]). The lipoid content is high (Shattock,[6] Vignes,[11] Cramer[9]). The pigment is apparently a lipochrome (Carlier and Evans[19]). Approximately 15 per cent of the gland is protein (Carlier and Evans[19]). Schoenheimer[20] (1942) has shown that lipids of the fat depots are not static or metabolically inert but are constantly subject to a variety of complex chemical reactions including synthesis, degradation, and interconversion. Fleischmann[21] stated that brown fat consumes more oxygen than white or yellow fat and Gomori[22] found that brown fat contained lipase whereas ordinary fat does not. Complete removal of this gland usually causes the death of the animal (Vignes[11]).

In hibernating animals, and some nonhibernating rodents, hibernal

masses have been found in the cervical region, axillae, between the scapulae and about the kidneys, and in the lumbar, inguinal, and gluteal regions (Hammar,[5] Auerbach[16]), all of which are presumably extensions from a large primary mass in the superior mediastinum. Homologous masses of adipose tissue in humans have been described by Merkel,[23] Hatai,[10] and Shattock.[6] Bonnot[24] studied the interscapular gland in human embryos from 3.2 to 28 cm. and also in newborn human infants and in adults, and stated that its length may be one-quarter that of the

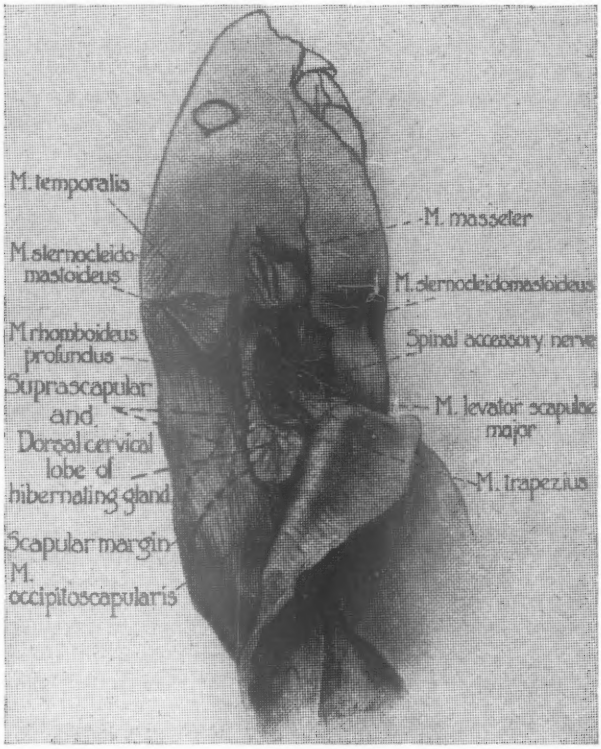


Text-Figure 2. Axillary lobe of hibernating gland of adult woodchuck.

crown-heel measurement of fetuses. Hatai[10] found the length of the gland to be up to 36 per cent of the length of the entire body.

The similarity of hibernating gland cells to the multilocular cells of developing (embryonic, immature, or juvenile) fat, sebaceous glands, and human brown fat (seen in cachexia or emaciation) is recognized. Neoplasms composed of incompletely differentiated fat cells, sometimes classified as atypical lipomas or liposarcomas, must be distinguished from hibernating gland tumors, the cells of which are not to be confused with immature fat cells sometimes encountered in lipomas. Whether a reversible transmutation can occur between the cells of the hibernating gland and ordinary fat is controversial. Hammar[5] was unable to produce unilocular fat cells in the hibernating gland by overfeeding rats. Hansemann[25] thought there were embryologic differences between ordi-

nary adipose tissue and hibernating gland and stated that one type does not change into the other. The possibility of these cells containing fine protein granules (Altmann's granules) from which fat may be synthesized has been expressed. Metzner[26] attempted to show that these granules are directly transformed into fat, but Bell[3] thought his observations



Text-Figure 3. Dorsal cervical part of developing hibernating gland in young woodchuck.

were far from convincing. Cramer[9] observed that these small granules increase in size and tend to push the nucleus to one side of the cell and to coalesce into one large drop. It is probably significant that the droplets and the granules are in the same arrangement. That the so-called hibernating gland is not a gland in the strict sense is realized, but its rôle as a possible source of internal secretion has been discussed. The designation "interscapular" is also probably unfortunate because it appears that only a small portion or none of the structure may actually lie between the scapulae.

In the development of the hibernating gland, a lymphoid and a vascular component have been mentioned. Hatai[10] stated that the "gland is composed of two entirely different constituent tissues; an outer fat tissue and an inner lymphoid structure .... which contains an abundant supply of blood and lymph sinuses and vessels." He interpreted the inner portion as a hemolymph node. Two of the reported neoplasms of this structure have been cystic and vascular, suggestive of cystic lymphangioma. The significance of these two components in relationship to tumor formation is suggestive but not clear.

The first case of neoplasm of the hibernating gland appears to have been reported in 1905 by Merkel,[39] who employed the term "adenoma of sebaceous gland." Merkel had been interested previously in fat of this type and had introduced the term "Fettpolster."[23] The reported cases of this tumor which are probably and possibly authentic are listed in Tables I and II. It is difficult to evaluate the meager descriptions of case reports, and therefore inaccuracies and injustices cannot be avoided. Rasor's[27] case in 1913 and Bonnel's[28] about 1 year later seem acceptable. Bonnel's case presentation was discussed by Louis Gery of the Institute of Anatomy at Strasbourg. In this discussion Gery reported another case and introduced the term "hibernoma," a name which has been accepted by later contributors. Gery, who did not independently report his case, compared the color of the tumor to that of the suprarenal gland and described the cells as fatty epithelioid cells.

TABLE I

Collected Cases of Hibernoma, Probably Authentic

Author	Year	Remarks
1. Merkel[39]	1905	Adenoma, sebaceous gland
2. Rasor[27]	1913	Female, age 25; back
3. Bonnel[28]	1914	Female; axilla
4. Gery[28]	1914	
5. Shaw[29]	1921	In exhibit in London, 1921
6. Inglis[12]	1927	Male, age 31; interscapular
7. Rochat[30]	1939	
8. Rochat[30]	1939	
9. Mosto and Radice[31]	1942	Female, age 37; axilla

Case 5 in Table I was mentioned in the notes of the annual meeting (1921) of the British Medical Association[29] in which reference was made to an exhibit arranged at Newcastle by A. D. Bernard Shaw containing a case from West London Hospital of lipoma glandulare (Inglis[12] also used this term), resembling hibernating gland and "thought to arise from a vestigial hibernating gland occasionally present in the human being." Rasor's[27] case report was combined with a contribution to the

granular synthesis of fat. The tumor which he reported was unusual in that it was a milky-white, soft, fluctuant neoplasm of the back and was composed to a considerable extent of unilocular fat cells, exemplifying all stages of transition between embryonal and adult fat. Reference to Rochat's[30] 2 cases appears in the minutes of the meeting of the Pathological Anatomical Society of the Netherlands held at The Hague in June, 1939. Rochat stated that he had had the opportunity to see 2 such cases which bore a perfect histologic resemblance to the hibernating organ of Inglis and that these have been called hibernomas. It is assumed that these were original cases not reported elsewhere. The anatomic sites of these two tumors were not stated. The title of the article by Mosto and Radice[31] was Hibernoma de Gery.

The cases which are included in Table II as possibly authentic are even more difficult to evaluate. Cases 1, 2, 3, and 5 were called sarcomas by the authors who reported them. The locations of cases 2, 3, 4, and 6 were not given. Camolle[33] "observed" 2 cases which perhaps should have been listed in Table I. They were studied by Uggeri,[34] who considered them to be similar to the cases of Rasor[27] and Merkel[39] (cases 1 and 2, Table I). Uggeri's case was placed in Table II because it was situated in

TABLE II

Collected Cases of Hibernoma, Possibly Authentic

Author	Year	Remarks
1. Mallory[32]	1918	Female; back; lipoblastoma; malignant?
2. Camolle[33]	1921	Observed 2 cases. Original(?). No description. Cited by Mosto and Radice.[31] Lipoblastic sarcoma. Studied by Uggeri.[34] Similar to cases of Rasor[27] and Merkel.[23]
3. Camolle[33]	1921	
4. Inglis[12]	1927	Female, age 6 wks.; hygroma with glandular fat areas
5. Börst[36]		Lipoblastic sarcoma, gluteal (noninfiltrating; nonmalignant)
6. Adair, Pack, and Farrior[37]	1932	Male, age 45; foam-celled tumor; xanthomyxolipoma
7. Uggeri[34]	1938	Female, age 57; leg, atypical lipoma

the leg; the hibernating gland has not been described in the leg or thigh. Inglis'[12] second case (case 4, Table II) might have been a composite tumor composed in part of multilocular fat and may have been related histogenically to the hibernating gland. Perhaps some of these tumors could best be called atypical lipomas, as suggested by Uggeri,[34] or pseudolipomas, as suggested by Lunghetti.[35]

The histologic similarity of hibernoma to granular cell myoblastoma

should be mentioned because it is reasonably possible that some of the former have been mistaken for the latter. Such possible confusion could be eliminated by the employment of fat stains on all soft tissue tumors composed of granular cells.

Report of Case

A Negress, 18 years old, was admitted (no. 46-11622) to the general surgical service of Detroit Receiving Hospital on September 9, 1946, because of a tumor of the right scapular region. She had been aware of a small lump in that region since early childhood but it had caused her no inconvenience until 1 year prior to admission. At that time the swelling had increased sufficiently to produce an unsightly bulge which could be seen easily through her clothing. The tumor had remained nontender at all times and the general health of the patient was good.

The past history was irrelevant and no other member of her family had recognizable tumors.

On admission the patient's temperature was 99° F.; pulse, 80; blood pressure, 135/80 mm. of Hg. Routine examination of the head, neck, chest, breasts, abdomen, and genitalia was noncontributory. Surgical scars were not present. Examination of the right scapular region revealed a soft, ovoid mass, measuring 12 cm. in diameter and elevated to 5 cm., dorsal to the right scapula. The mass was freely movable, nontender, slightly lobulated, and superficial vessels could be seen beneath the skin surface. There was a noticeable increase in local skin temperature.

Radiographic examination of the area indicated that there was no attachment of the tumor to the underlying bone.

Laboratory findings were as follows: Serologic tests for syphilis, negative; hemoglobin, 10.5 gm. per 100 cc. of blood; leukocytes, 4500 per cmm., with 68 per cent polymorphonuclear neutrophils (64 per cent segmented and 4 per cent nonsegmented), and 32 per cent small lymphocytes. Upon routine chemical and microscopic examination the urine was normal.

Surgical excision of the tumor was accomplished with considerable difficulty because of excessive vascularity. The mass was intimately attached to the deep fascia and muscle. The postoperative course was uneventful.

Upon gross pathologic examination the specimen was found to be a partially encapsulated, soft, discoid mass measuring 10 by 8 by 5 cm. and weighing 315 gm. Small fatty tabs were adherent to the surface. The mass was composed of fairly uniform, parallel bundles measuring from 2 to 5 mm. in diameter, which were separated with ease. The color varied from yellowish to reddish brown. After fixation in formalin the color was grayish brown and then the appearance was that of boiled muscle.

Histologically, the tumor was composed of large cells, most of which varied from 20 to 50 μ in diameter, with centrally placed nuclei in which the chromatin was finely and coarsely distributed. There were 1 to 3 nucleoli per nucleus, but the nucleolar-nuclear ratio rarely exceeded 1:4. The cytoplasmic-nuclear ratio varied from 4:1 to 10:1. The smaller cells contained fine and coarse granules; the larger cells contained small vacuoles or locules. Both the granules and locules stained black with

osmic acid and both were sudanophilic. In some multiloculated cells there appeared to be condensation or coalescence of smaller into larger locules, and when this became extreme the nucleus was then eccentrically or peripherally placed. A few unilocular fat cells were present which measured from 50 to 100 μ in diameter and, while it might be argued that these represented extrinsic adipose tissue, there seemed to be evidence of a transition from multilocular to unilocular fat cells. The tumor was divided into lobules, within which there was a small amount of finely divided reticulum. In sections cut perpendicularly to the above-described bundles the lobules were separated by loose areolar tissue.

*Report on Fat Analysis.** The tissue was dried in a vacuum oven at 45° to 50°C. for 2 days. Drying was continued over calcium chloride for several days and the final weight was 27 per cent of the original specimen, indicating that the water content was approximately 73 per cent. The dried tissue was then extracted with alcohol and ether for 12 hours and this process was repeated several times. Sixty per cent of the dry weight was thus extracted. A portion of moist tissue specimen of similar size was refluxed with hot alcohol for 24 hours, the alcohol decanted into a weighed flask, and the dried residue weighed. This process was repeated several times with alcohol and then with ether until no fat was extracted and the remaining tissue was white and dry and could be ground and powdered. The extracted fat was 17.3 per cent of the net weight. The average of these determinations was 16.85 per cent, which represented the fat content of the tumor. The melting point of the fat was between 34° and 35°C. and the iodine number was 65.

The fat was then separated into the saponifiable and unsaponifiable fractions as described by Sperry and Bloor.[38] Of the total fat, over 80 per cent was saponifiable and 12 per cent unsaponifiable.

Summary

The development of the so-called hibernating gland from persistent and permanent brown multilocular fat has been firmly established. Neoplasms developing in a homologous structure in humans have been occasionally reported since 1905 and now constitute an oncologic entity. The reported cases of hibernoma, by whatever name they have been designated, are difficult to evaluate, but 9 seem to be reasonably authentic, while 7 others are possibly acceptable.

The histologic study of the neoplasm in the case reported indicates that there is a transition from the "granular" cells to multiloculated cells

* Performed by Ruth M. Davis, Ph.D., Department of Physiological Chemistry, Wayne University.

and even to unilocular fat cells. The fine granules sometimes referred to as Altmann's granules in the smaller neoplastic cells are apparently minute droplets of fat.

Care should be taken not to confuse this neoplasm with atypical lipomas, low-grade liposarcomas, or granular cell myoblastomas.

While "hibernoma" is admittedly a somewhat undesirable term, it possesses priority, for Gery, in 1914, gave an otherwise nameless tumor this name. The term refers not to hibernation but to the relationship of the neoplasm to hibernal fat. An attempt to find a more descriptive or appropriate name might easily lead to cumbersome terms and confusion. A challenge obviously exists for someone with a flair for terminology to improve or remedy this weakness in nomenclature.

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DESCRIPTION OF PLATES

PLATE 66

FIG. 1. Hibernoma. External and cut surface of the gross specimen. The tumor measured 10 by 8 by 5 cm.

FIG. 2. Photomicrograph illustrating the lobulated architecture of the hibernoma. Hematoxylin and eosin stain. × 200.

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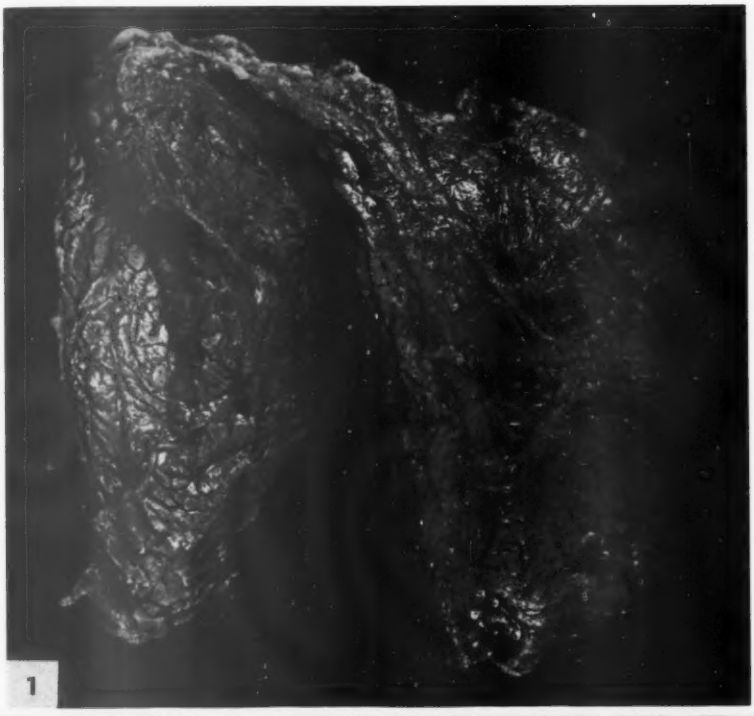
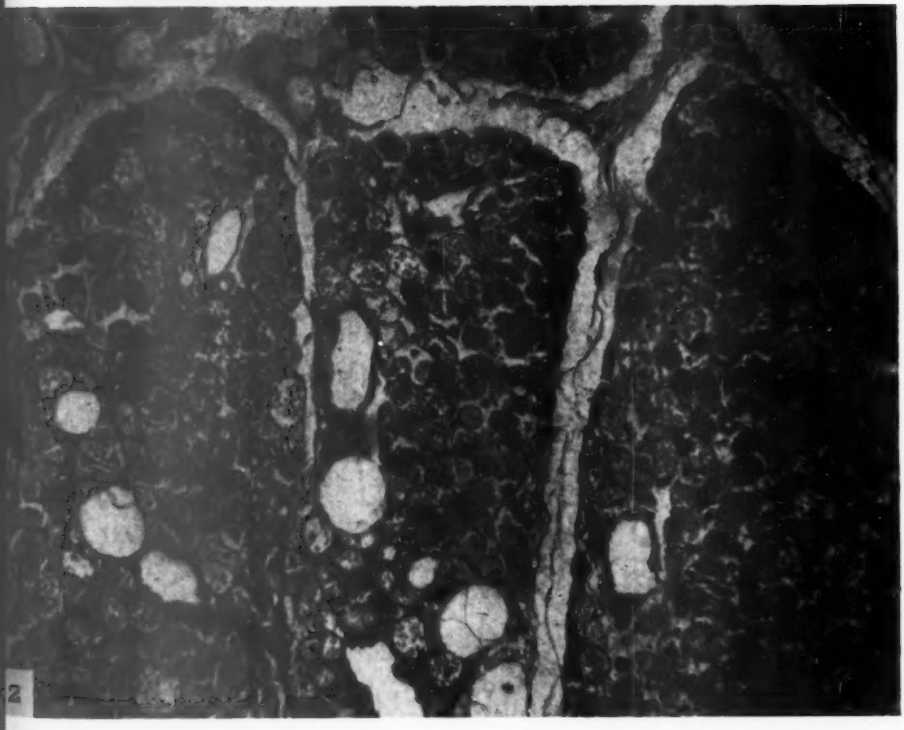


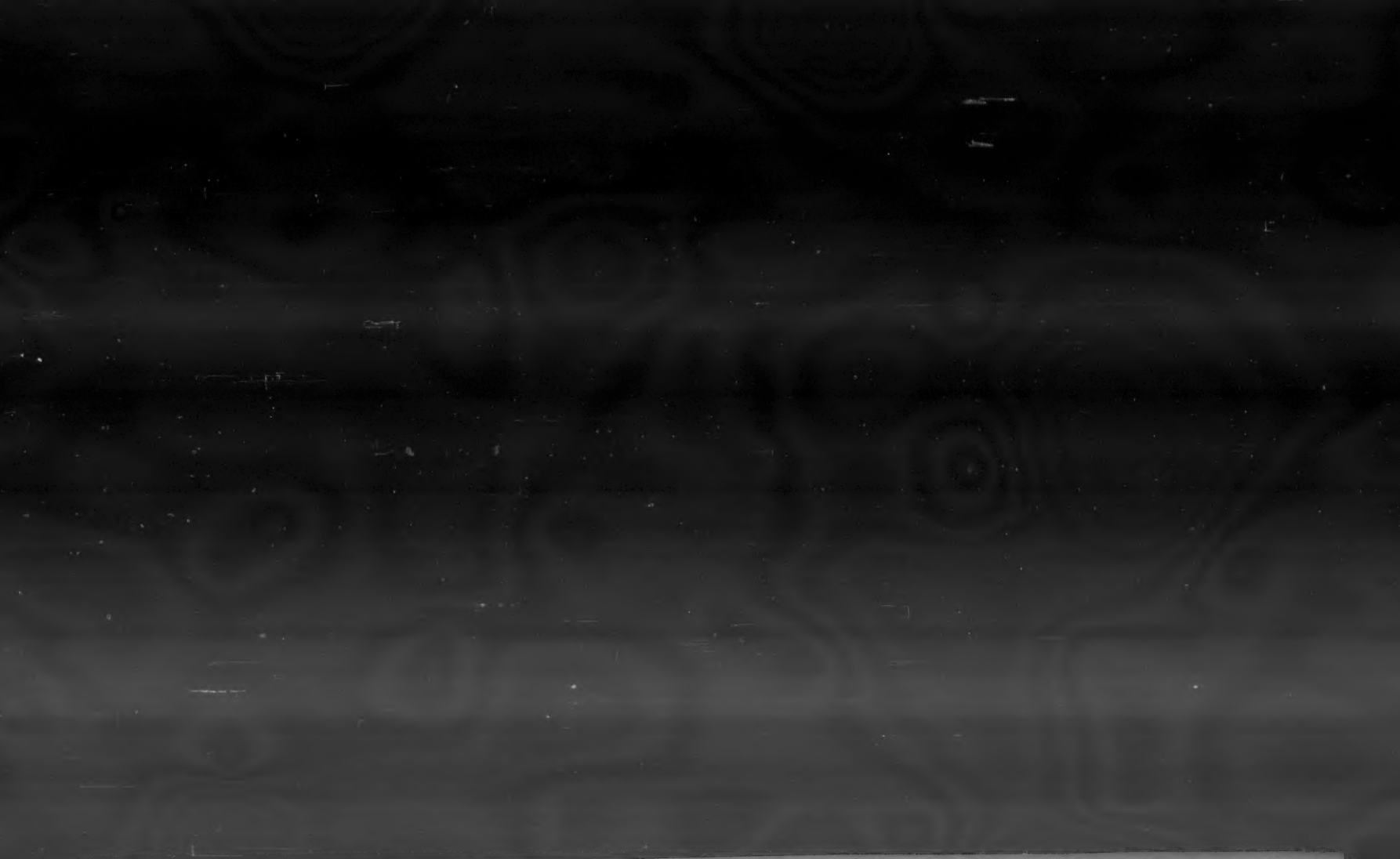


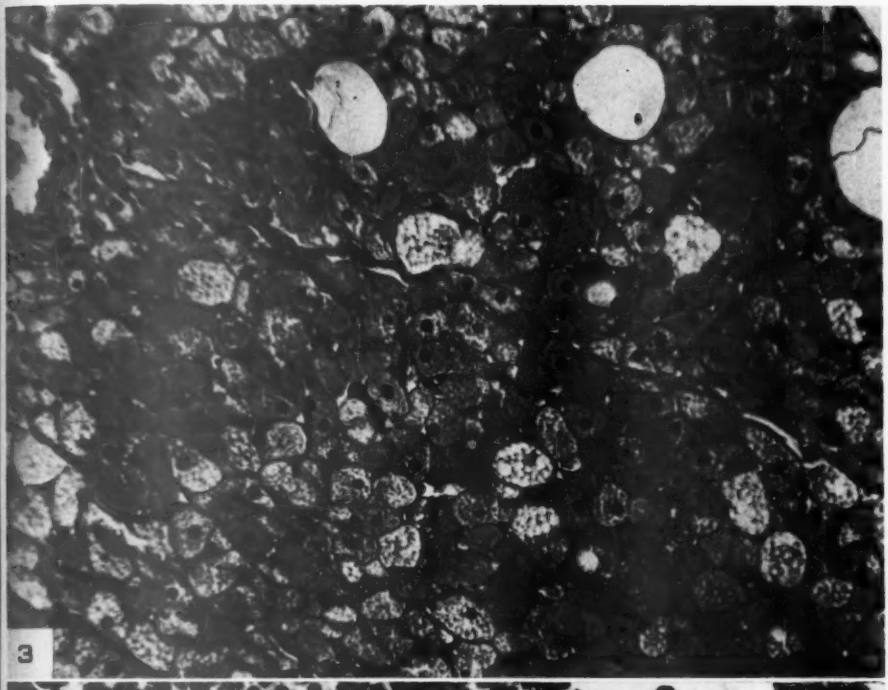
Brines and Johnson Hibernoma, a Special Fatty Tumor

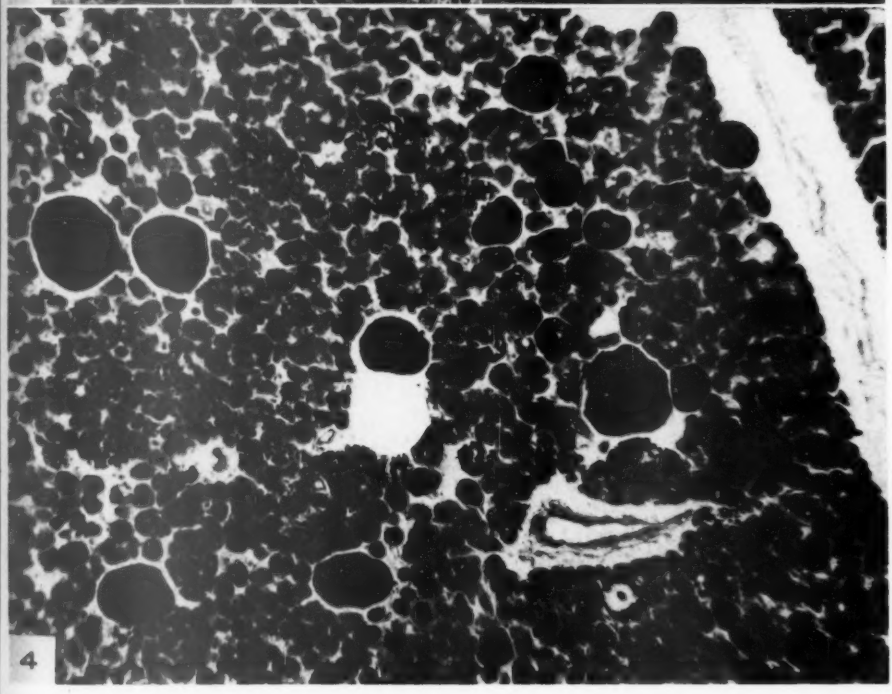
PLATE 67

FIG. 3. Cell size depends upon the fat content of the cytoplasm. Transition from finely granular cells, through multiloculated cells, and finally to adult fat cells is indicated. Masson's stain. × 300.

FIG. 4. The varying degrees of fat content, and the size and distribution of the locules of fat are indicated. The fine granules are fat-positive. Osmic acid stain. × 200.







Brines and Johnson Hibernoma, a Special Fatty Tumor

ARTERIOSCLEROTIC LESIONS IN PYRIDOXINE-DEFICIENT MONKEYS *

JAMES F. RINEHART, M.D., and LOUIS D. GREENBERG, Ph.D.
(From the Division of Pathology, University of California Medical School, San Francisco 22, Calif.)

Adaptation of the rhesus monkey (*Macaca mulatta*) to a synthetic diet has opened the way to a re-exploration of the metabolic and structural defects resulting from single vitamin deficiencies. Such studies are particularly desirable in a primate, with metabolic processes similar to those of man. We have reported briefly our observations on thiamine[1-4] and folic acid[5,6] deficiency.

During the past 2½ years we have undertaken a systematic study of pyridoxine deficiency in the rhesus monkey. Observations have been made on the blood picture, blood and tissue contents of pyridoxine, and the metabolism of tryptophane, in addition to detailed morphologic examinations of tissues. This report is concerned with the pathologic findings, with particular reference to the degenerative and proliferative changes observed in arteries. A preliminary note of these observations recently has been made.[7]

EXPERIMENTAL PROCEDURE

The basal diet was a modified M-3 diet[8] and contained powdered sucrose, 73 parts; vitamin-free casein, 18 parts; Hawk and Oser salt mixture, 4 parts; and corn oil, 2 parts. It was compressed in 2 gm. tablets following granulation and the addition of 1 per cent calcium stearate. The tablets were fed *ad libitum*. A tablet containing the following vitamin supplements was fed daily: nicotinic acid, 5 mg.; riboflavin, 1 mg.; thiamine chloride, 0.5 mg.; calcium pantothenate, 3 mg.; choline dihydrogen citrate, 100 mg.; paraminobenzoic acid, 100 mg.; inositol, 100 mg.; ascorbic acid, 25 mg., with sufficient powdered sugar to produce a tablet weighing 1.5 gm. In addition the monkeys received by mouth, twice weekly, 5 drops of vitamin A and D concentrate (100,000 international units of vitamin A and 10,000 international units of vitamin D per gm.), 385 μg. of pteroylglutamic acid,† 35 μg. of biotin, and 5 drops

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† Two of the monkeys were started on one-half this intake of pteroylglutamic acid. This is equivalent to a daily dose of 55 μg. and was originally reported to be adequate (Totter and associates.[9]). Dr. Totter later informed us that this intake was not optimal so we increased it to 110 μg. per day. Our own experience indicates that this is a more nearly optimal intake.

of mixed natural tocopherols* once a week. Control monkeys also were provided with 3.5 mg. of pyridoxine hydrochloride twice a week.

Observations on 5 pyridoxine-deficient monkeys are reported in this study, 4 immature males and one female ranging in weight from 2375 to 3200 gm. During an initial control period the animals were given the diet with the complete vitamin supplement. In the absence of complicating influences such as infection, the animals ate the diet surprisingly well, gained weight, and remained vigorous and active. In fact, one monkey, maintained on the diet for 42 months, is one of the most vigorous, healthy monkeys we have had in the laboratory. After the control period, pyridoxine was removed from the diet. The animals ordinarily continued to eat well and gained weight for 2 to 3 weeks, after which the food consumption began to fall off and the animals lost weight. In most instances there was a gradual continued weight loss. Aside from the decreased food consumption, gradual weight loss, and diminished vigor the animals showed little change in appearance until the depletion had been carried for a period of 5 to 6 months. Then they became unkempt and somewhat sluggish unless disturbed, when they seemed hyper-irritable and "jumpy." Convulsions have not occurred. The hair usually became thinner and lighter; hair growth was almost completely arrested. Some animals showed edema of the eyelids and late in the course of the experiment most had developed some fissuring of the epithelium of the palms of the hands and feet. All developed a moderate leukopenia and anemia. Observations on the pyridoxine content of the blood indicated that this paralleled the intake. During the control period in which the animals received 1 mg. of pyridoxine daily, the pyridoxine values of the blood averaged 11 μg. per 100 cc., while during depletion the values ranged from 1.5 to 4 μg. per 100 cc.

Pathologic Findings

To date, pathologic examinations have been made on 5 animals which had been subjected to pyridoxine deficiency for periods ranging from 5½ to 16 months. The anemia, leukopenia, and associated bone marrow alterations occurring in pyridoxine deficiency will be the subject of a separate report. Significant lesions were observed in the livers of 2 animals. One animal, subjected to pyridoxine deficiency for 16 months, showed a faun-colored, nodular liver in which degenerative changes and regenerative hyperplasia were evident. The other animal, deficient for 7 months, showed a mild periportal and diffuse fibrosis. It is evident that more extensive study will be required to determine the incidence and significance of this finding.

* Nopco Chemical Co., Harrison, N. J.

This report is concerned primarily with the arterial lesions, which are the most constant and prominent abnormality encountered. Sclerotic lesions have developed in the arteries of each of the 5 animals subjected to pyridoxine deficiency. In the 2 animals which were examined at the end of 5½ months the changes, while definite, were not extensive. In one (R.47.441), intimal fibrosis was present in arterial branches in the pancreas (Fig. 1), kidney (Figs. 2 and 3), and serosa of the colon (Fig. 6). The other animal showed definite but minor changes in the branches of the renal arteries and in the coronary arteries. Similar lesions in the arteries of the kidneys and pancreas were seen in an animal subjected to deficiency for 7 months. More widespread and advanced arteriosclerotic lesions have developed in 2 animals which were maintained on a pyridoxine-deficient diet for 13 and 16 months, respectively. In these animals lesions were prominent in the coronary arteries (Figs. 7, 9, 10, 11, and 12), as well as in arteries of the kidney (Figs. 4 and 5), pancreas, and elsewhere. Particularly advanced changes were present in arteries of the testicle of one animal (Fig. 8). It was somewhat surprising to see an advanced "arteriosclerosis" in the vessels of an immature testis. Small plaques of fibrous tissue developed in the intima of the arteries mentioned, which bore a close resemblance to human lesions of arteriosclerosis. The new-formed fibrous tissue plaques lay between the endothelium and the internal elastic lamina. In some instances there were minute blister-like structures where the endothelium had separated from the underlying fibrous tissue (Figs. 9 and 10). Occasionally "splitting" and duplication of the internal elastic lamina occurred (Fig. 4). The lesions were well demonstrated by a combination of Weigert's elastic tissue and van Gieson's stains. They tended to be patchy in distribution (Fig. 11), a feature characteristic of arteriosclerosis in man. Occurrence of the lesions at bifurcation points (Fig. 12) was another feature of the experimental lesion common to the human disease. Lesions in branches of the renal vessels showed a relatively coarse, intimal, collagenous sclerosis. In the coronary vessels the intimal fibrosis consisted of a more delicate collagen and the plaques appeared more edematous. Detailed examination of the aorta was not made. Gross lesions were not noted, but microscopic patches of edematous intimal fibrosis of the type encountered in other vessels were demonstrated in the 2 animals maintained on the deficiency for over 1 year. Likewise, while detailed study of the blood vessels supplying the extremities was not made, analogous lesions were found in those vessels which were examined.

The pathogenesis of these experimental arteriosclerotic lesions has not been determined, but observations suggest that there is an alteration in the connective tissue ground substance of the intima. In sections

stained with hematoxylin, slightly basophilic, mucoid-appearing material has been seen lying between the proliferating connective tissue cells. This, with the "blister-like" separation of the endothelium seen in some instances, suggests some fault in a binding substance. This might be related to the defect in protein metabolism described in pyridoxine deficiency in other animals[10] and recently shown by us to occur in the monkey.[11] There seems no question that the arterial lesions are related to pyridoxine deficiency. Such lesions have not developed in monkeys on the same dietary regime but subjected to other dietary deficiencies and showing equal degrees of inanition. The experimental lesions which have been described have a close resemblance to arteriosclerosis as it occurs in man.

It is of interest to note that Virchow, according to Aschoff,[12] considered the first change in the atherosclerotic process to be a "certain loosening of the connective-tissue ground substance" of which the arterial intima is for the most part composed. "This swelling of the ground substance . . . . is recognized microscopically by the increased width and homogeneity of the connective-tissue spaces." Aschoff adds, "Pari passu with the thickening and transformation of the ground substance, the connective-tissue cells of the intima undergo changes. They enlarge in all dimensions, divide and form localized thickenings." Other students of the disease have emphasized the fundamental proliferative aspect of the arteriosclerotic process. It would seem likely that imbibition of fluid and colloidal substances such as cholesterol from the blood plasma would be favored by such a pathologic process.

Discussion

Experimental attempts at production of arteriosclerotic lesions in primates have been notably unsuccessful. While Fox[13] has recorded the spontaneous occurrence of arteriosclerosis in certain captive primates, he has not observed the condition in *Macaca rhesus*. It is noteworthy that prolonged feeding of cholesterol has failed to produce significant lesions in the monkey.[14] This is, in fact, a potent argument against the cholesterol theory as a major influence in the pathogenesis of arteriosclerosis in man. As far as we know, the lesions here reported are the first of this type that have been described as resulting from deficiency of a specific food factor. The observation gains added significance in that the lesions have been produced in a primate which, in nutritional metabolism, is closely related to man. Furthermore, the lesions have resulted from an experimental circumstance that might occur in man.

While it has not yet been shown conclusively that pyridoxine is required by man, certain clinical observations strongly suggest that it is.[15]

It would be most surprising if this substance were not essential for normal metabolism in man inasmuch as it has been found to be required in the diet of all animals so far investigated.[10] Prior studies in animals have been concerned primarily with the influence of deficiency on the blood picture and on iron metabolism.[10] Vascular lesions have not been described heretofore. It is noteworthy that pyridoxine deficiency is in essence a chronic deficiency, relatively slow in evolution and without distinctive external manifestations. Such a deficiency state would be one particularly difficult of clinical recognition. It remains to be determined to what extent pyridoxine deficiency may occur in man and, if it does occur, what influence it may have in the pathogenesis of arteriosclerosis.

Summary

Pyridoxine deficiency in the rhesus monkey produces sclerotic lesions in arteries bearing close resemblance to those of arteriosclerosis in man.

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DESCRIPTION OF PLATES

PLATE 68

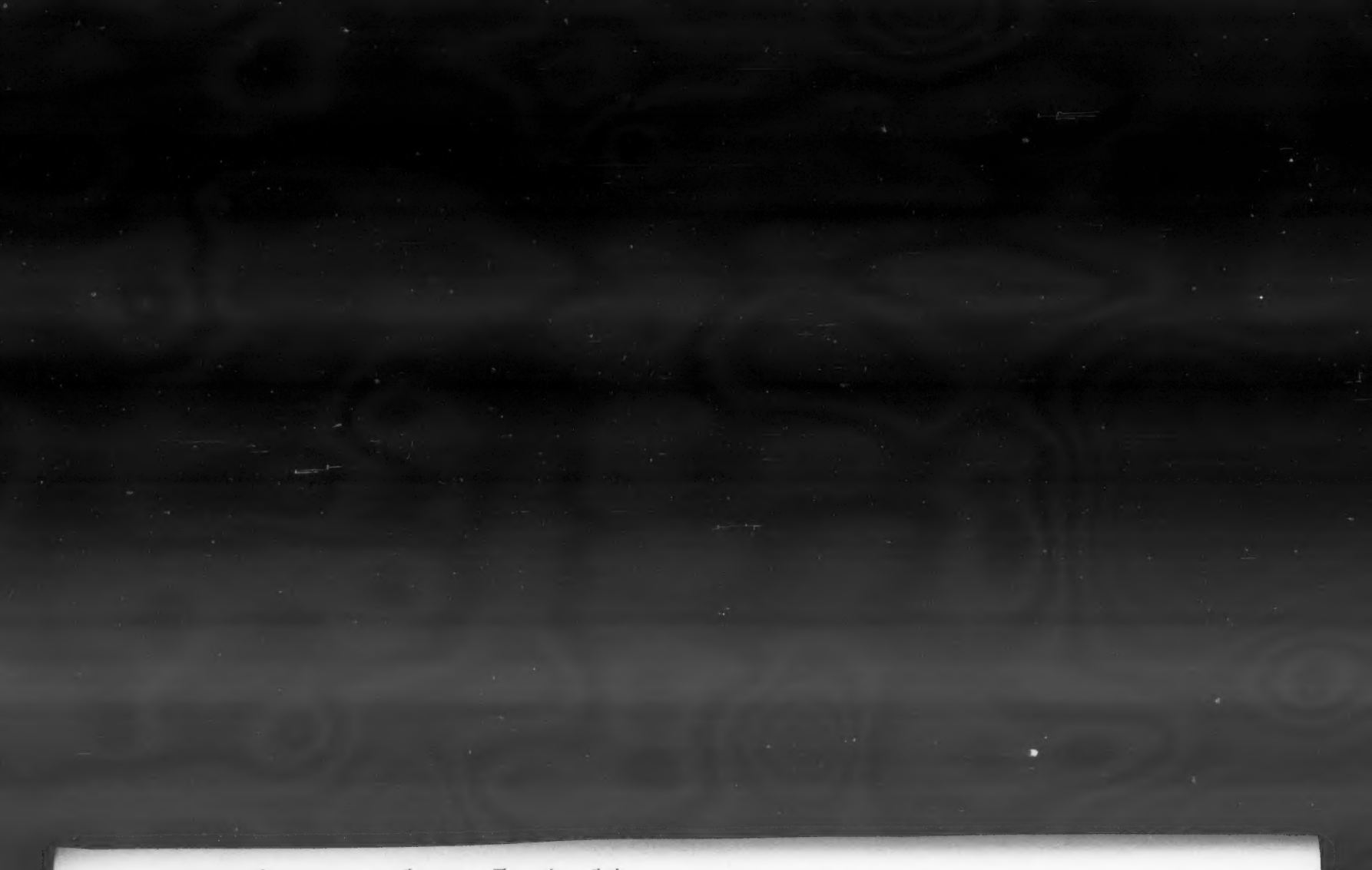
All photomicrographs are of sections stained by a combination of Weigert's elastica and van Gieson's methods.

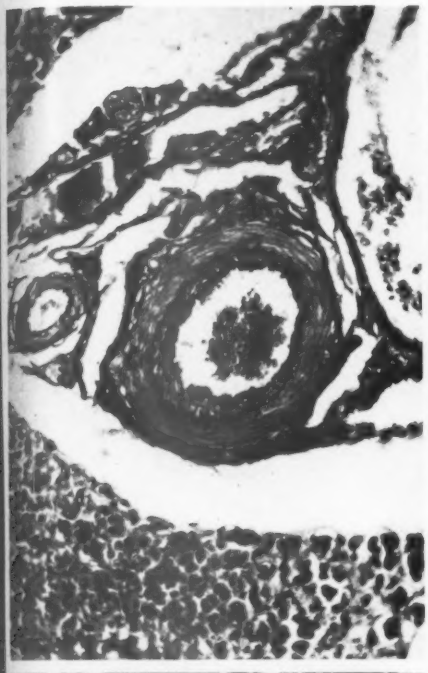
FIG. 1. Intimal fibrosis in a branch of a pancreatic artery. Monkey R.47.441: pyridoxine deficiency, 5½ months. × 162.

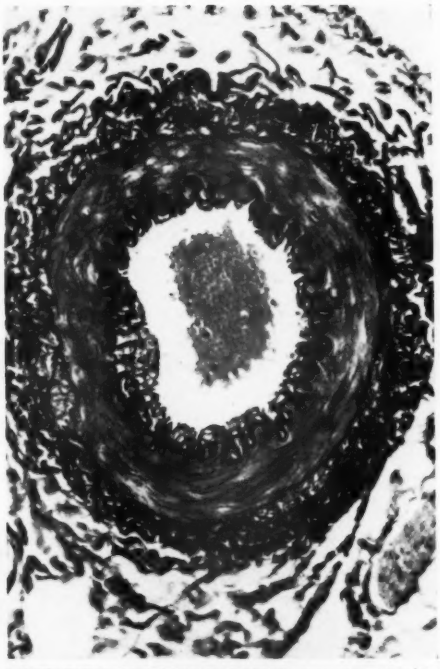
FIG. 2. Early eccentric collagenous intimal thickening in a major branch of a renal artery. Monkey R.47.441: deficiency, 5½ months. × 80.

FIG. 3. Marked collagenous intimal thickening in a small branch of a renal artery. Monkey R.47.441: deficiency, 5½ months. × 162.

FIG. 4. Intimal collagenous sclerosis with reduplication of internal elastic lamina in a small branch of a renal artery. Monkey R.47.199: deficiency, 16 months. × 325.







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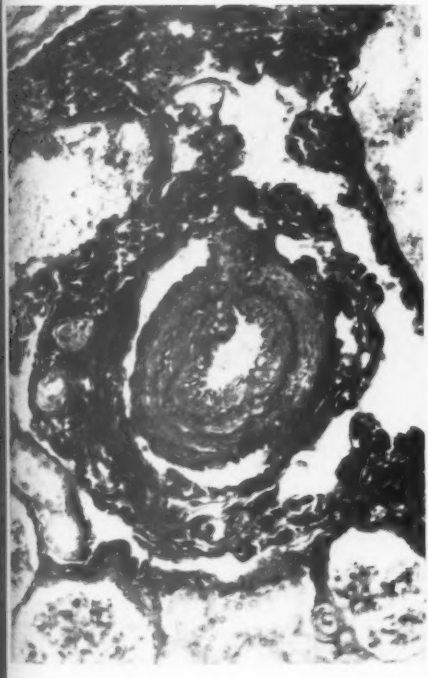

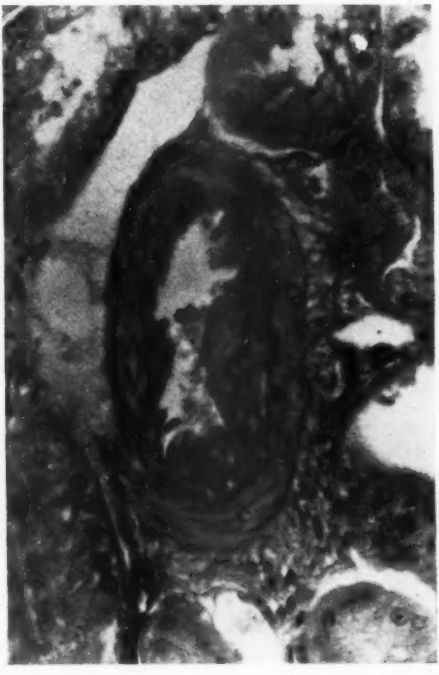

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PLATE 69

FIG. 5. Intimal fibrosis in a small branch of a renal artery. Monkey R.47.1261: deficiency, 13 months. × 160.

FIG. 6. Uniform intimal fibrosis in small arteries in the serosa of the colon. Monkey R.47.441: deficiency, 5½ months. × 80.

FIG. 7. Early eccentric intimal fibrous plaque in a branch of a coronary artery. Monkey R.47.1261: deficiency, 13 months. × 160.

FIG. 8. Marked occlusive intimal fibrosis in an artery of the testicular tunic. Of note is the delicate edematous-appearing collagen. Monkey R.47.199: deficiency, 16 months. × 80.

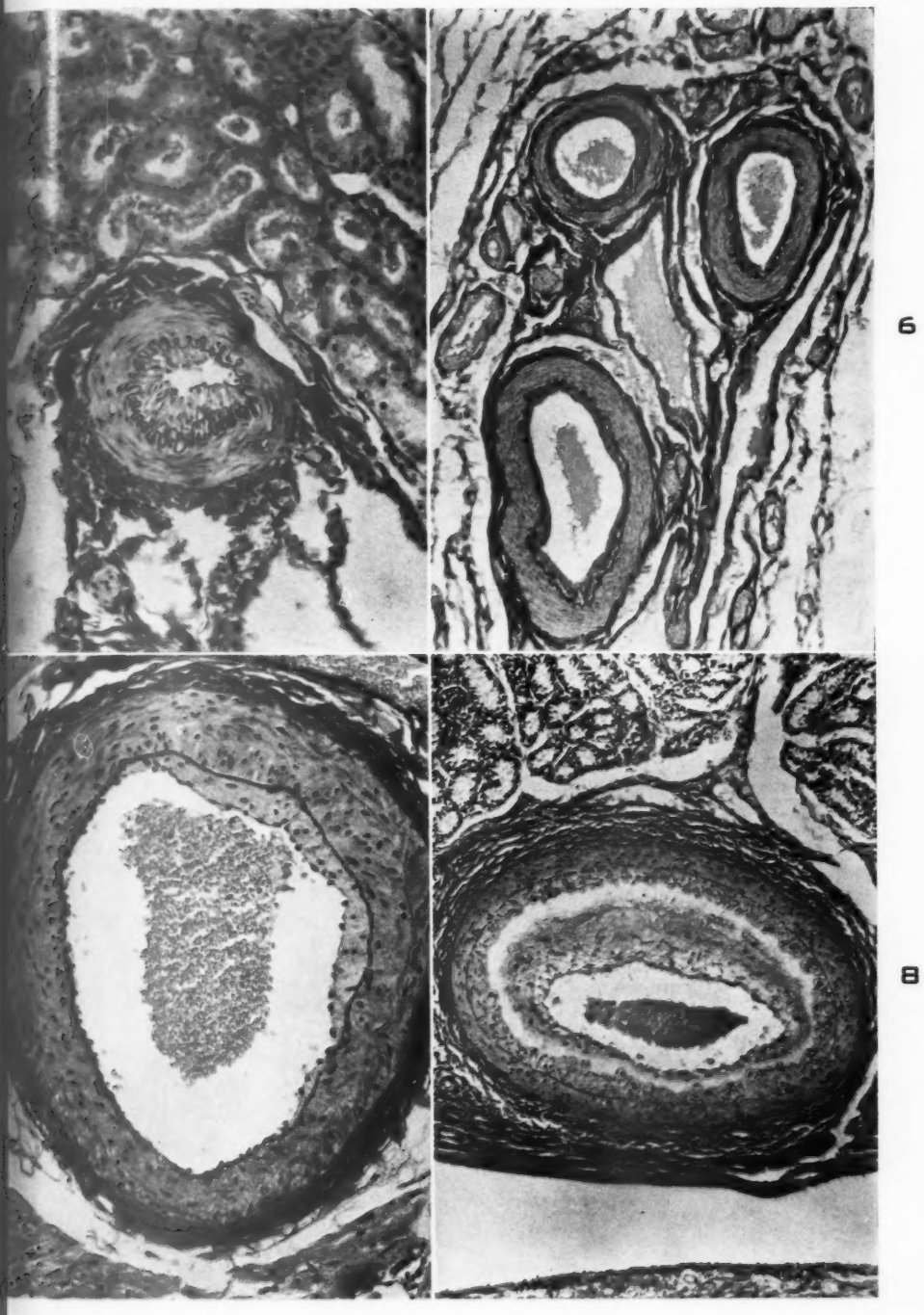

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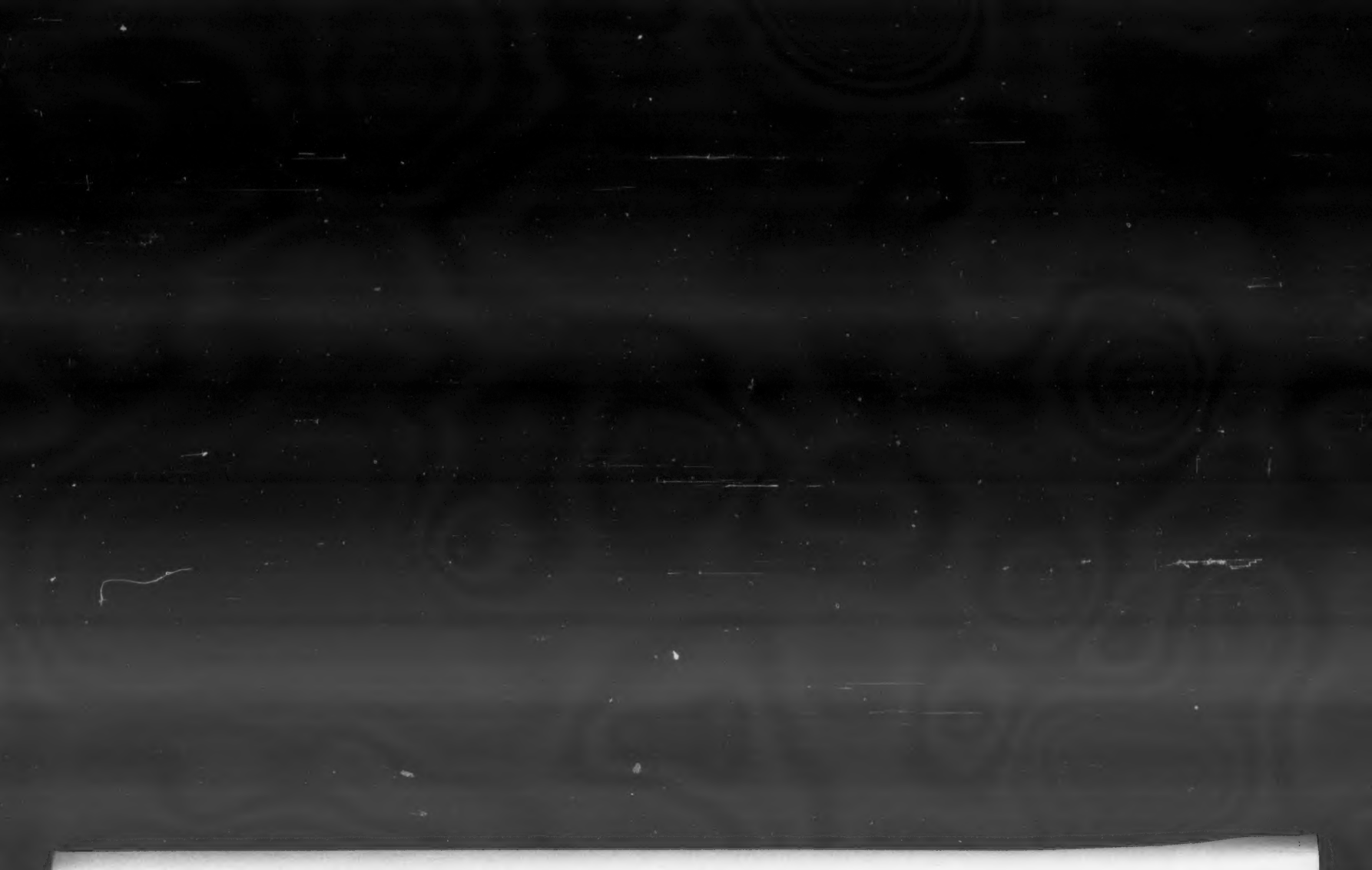
PLATE 70

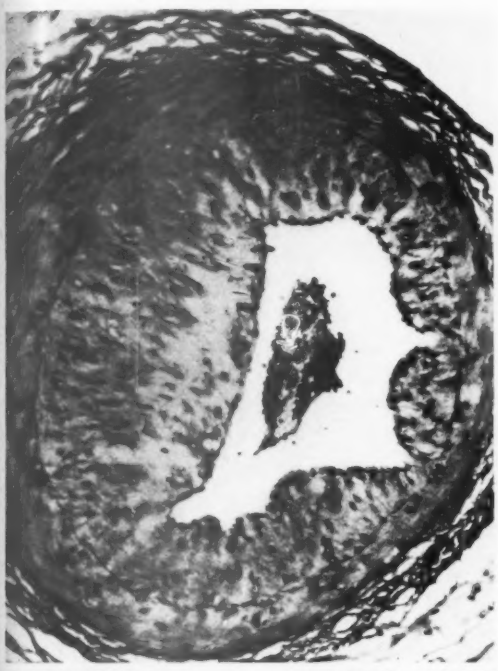
FIG. 9. Eccentric edematous hyperplasia of intimal fibrous tissue in a branch of a coronary artery. Monkey R.47.199: deficiency, 16 months. × 162.

FIG. 10. Major coronary artery showing marked hyperplasia of intimal fibrous tissue. Monkey R.47.199: deficiency, 16 months. × 80.

FIG. 11. Major coronary artery showing eccentric patches of intimal fibrosis. Monkey R.47.1261: deficiency, 13 months. × 80.

FIG. 12. Branch of a coronary artery at bifurcation zone. Of note is an evident predilection for fibrous intimal plaques at this site. Monkey R.47.1261: deficiency, 13 months. × 80.





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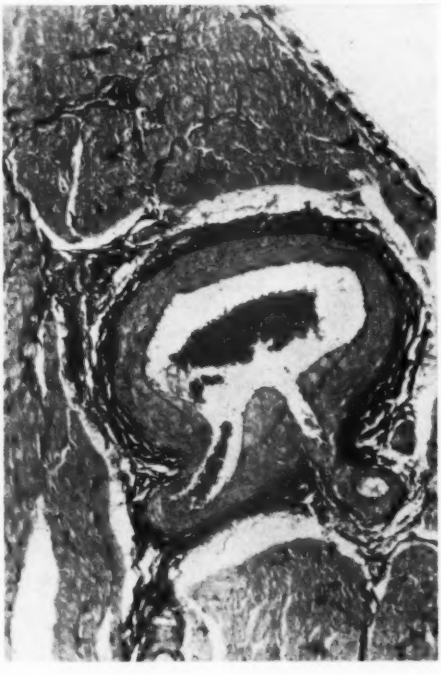

12

THE MORPHOLOGY, ANDROGENIC FUNCTION, HYPERPLASIA, AND TUMORS OF THE HUMAN OVARIAN HILUS CELLS *

WILLIAM H. STERNBERG, M.D.

(From the Department of Pathology, School of Medicine, Tulane University of Louisiana and the Charity Hospital of Louisiana, New Orleans, La.)

The hilus of the human ovary contains nests of cells morphologically identical with testicular Leydig cells, and which, in all probability, produce androgens. Multiple sections through the ovarian hilus and mesovarium will reveal these small nests microscopically in at least 80 per cent of adult ovaries; probably in all adult ovaries if sufficient sections are made. Although they had been noted previously by a number of authors (Aichel,[1] Bucura,[2] and von Winiwarter[3,4]) who failed to recognize their significance, Berger,[5-9] in 1922 and in subsequent years, presented the first sound morphologic studies of the ovarian hilus cells. Nevertheless, there is comparatively little reference to these cells in the American medical literature, and they are not mentioned in standard textbooks of histology, gynecologic pathology, nor in monographs on ovarian tumors (with the exception of Selye's recent "Atlas of Ovarian Tumors"[10]).

The hilus cells are found in clusters along the length of the ovarian hilus and in the adjacent mesovarium. They are, almost without exception, found in contiguity with the nonmyelinated nerves of the hilus, often in intimate relationship to the abundant vascular and lymphatic spaces in this area. Cytologically, a point for point correspondence with the testicular Leydig cells can be established in terms of nuclear and cytoplasmic detail, lipids, lipochrome pigment, and crystalloids of Reinke.

This paper presents a morphologic study of the ovarian hilus cells, a report of 2 cases of masculinizing tumors of these cells, as well as 2 instances of hilus cell hyperplasia associated with masculinization. Additional data concerning the functional significance of the cells and their response to chorionic gonadotropin are recorded.

TERMINOLOGY

A number of names have been applied to the hilus cells. Berger[5-9] referred to them as "sympathicotropic" cells. Kohn[11] used the term "extraglandular interstitial cells" or "extraglandular Leydig cells."

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Neumann,[12] Wieser,[13] and others have referred to them as "hilus cells."

The adjective "sympathicotropic" was applied by Berger because of the almost constant anatomic relationship to the nonmyelinated nerves of the ovarian hilus and mesovarium. However, the term has a physiologic connotation which is perhaps unwarranted in our present state of ignorance. The term "interstitial cell" is likely to be confusing. Interstitial cells of the ovary, apparently derived from theca interna, are prominent in certain animals but there is disagreement as to their existence in humans. In any case they appear unrelated to hilus cells. The term "extraglandular Leydig cell," though a bit cumbersome, has much to commend it. The cells are morphologically identical with Leydig cells and are undoubtedly androgenic. Nevertheless, absolute identity has not been proved; and it may be that the relationship to nerves indicates an additional function, not yet elucidated, which may establish the cells as cousins rather than sisters to the Leydig cells of the testis.

I shall refer to these cells as "ovarian hilus cells." Fortunately, none of the other numerous cell types resident in the ovarian hilus has been so designated. The term suffers, of course, from lack of specificity, but that perhaps may be an advantage at present.

RESUMÉ OF LITERATURE

There are scattered studies of ovarian hilus cells in the earlier literature by authors (Aschoff,[14] Bucura,[2] von Winiwarter,[3] and others) who considered them part of the chromaffin system. Since 1922, Berger,[5-9] in a series of studies, has presented convincing morphologic evidence of the similarity of these cells to testicular Leydig cells, referring to them as sympathicotropic cells. A lively controversy between the proponents of these two theories existed for many years. Reference may be made to the papers of de Winiwarter,[15-17] the stoutest proponent of the "paraganglion cell" theory, as well as to those of Wallart[18] and Joachimovits.[19] Since Berger's initial studies, most investigators have agreed with his conclusions (Kohn,[11] Pawlowski,[20] Brannon,[21] Wieser,[13] Barrozo do Amaral[22]).

Particular reference is made to the study by Kohn[11] (who, incidentally, discovered the pheochrome system). After a morphologic analysis he rejected the possibility that the ovarian hilus cell belongs to the chromaffin system. Most investigators, including myself, have been unable to demonstrate a chromaffin reaction in these cells. It is altogether likely that some who have reported chromaffin staining were misled by the brown lipochrome pigment normally present in many of the cells.

In the human newborn the identification of cells adjacent to the ovary is complicated, since true pheochrome cells also may be present, particularly in the broad ligament. Chromaffin tissue in the embryo, after separating from the sympathico-chromaffin anlagen, may be closely associated with the wolffian body. Zuckerkandl[23] noted that chromaffin tissue may be found in the fetus and newborn along the ureters, in the broad ligaments, retroperitoneal tissues, and elsewhere. However, these nests are morphologically distinct from the hilus cells and do show a chromaffin reaction. Berger[7] has reported the simultaneous presence of cells of both types in the newborn. Pheochrome nests about the ovary, however, are quite rare in the adult since in the human there is a general involution of chromaffin tissues shortly after birth.

Cell nests identical with the ovarian hilus cells have long been known to exist in the male gonad outside of the testis proper. Verocay,[24] in 1915, first studied these, and they have since been noted by numerous authors.[25,26] They may be found in the tunica albuginea, the hilus of the testis, the region of the rete testis, along the spermatic cord, and elsewhere. They have, furthermore, precisely the same relationship to nonmyelinated nerves as do ovarian hilus cells, and in fact are histologically indistinguishable from them. Berger[5,8] has studied these extratesticular Leydig cells extensively, as have Wieser,[13] Kohn,[11] Nelson,[27] and others.

Kohn[11] studied ovarian hilus cells in the newborn, and noted their prominence in an anencephalic infant. He found them as late as the 77th year of life. Brannon,[21] who studied a large series of ovaries, found abundant hilus cells in ovaries of 4 pregnant women, all cases of toxemia.

Morphology of Ovarian Hilus Cells

Over 100 ovaries (singly or in pairs) taken from routine surgical and autopsy material were studied. Since the hilus cells cannot be distinguished grossly and since they are distributed in irregularly scattered clumps, they may be absent from some sections cut through the hilus of an ovary but present in others. However, using a limited number of sections (4 to 6, on an average) hilus cells were identified in approximately 80 per cent of the adult ovaries studied. In the ovaries of 12 adult women a more thorough search was made and hilus cells were found in all cases. It is altogether probable that some of these cells are present in all adult ovaries. In general more hilus cells were found per section when the ovarian hilus and mesovarium were sectioned longitudinally after fixation.

DISTRIBUTION

There was considerable variation both in the number and distribution of the ovarian hilus cells. They were present in the hilus and mesovarium, being somewhat more numerous at the lateral and medial poles of the hilus, and particularly near the junction of the ovarian ligament and ovary. Cell nests were seen frequently in the areolar tissue of the hilus close to its well defined junction with the ovarian stroma. However, nests were seen often much deeper in the mesovarium. Cell nests might be found close to the rete ovarii although no constant anatomic relationship was apparent. Occasional nests were incorporated in the ovarian stroma, but these were almost always close to the hilus and appeared to represent extensions of the hilus into the ovary proper.

The cells occurred in aggregates that varied considerably in size, shape, and number of cells. Irregularly shaped cell clusters often partially surrounded vessels and nerves in the vicinity. Unlike the occasional adrenal rests of the region, they never were encapsulated or delimited sharply from the surrounding tissues, but rather seemed indigenous to the hilar stroma.

CYTOLOGY

The remarkable range of morphologic variations in the testicular Leydig cells is well known to all who have studied them. They vary from cells indistinguishable from the fibroblast, through elongate, polygonal, and oval forms containing an inconstant variety of intracytoplasmic inclusions, to rare giant multinucleated types. This protean variation in form might appear to offer difficulties in establishing the morphologic identity of hilus and Leydig cells. Actually, the reverse is the case, since the ovarian hilus cells reveal precisely the same types of morphologic variation.

The majority of ovarian hilus cells measured from 14 to 25 μ in diameter, although occasional larger forms were seen. Frequently each cell in a group was separated from its neighbor by a delicate collagenous fibril, as seen often with testicular Leydig cells. The cells for the most part were polygonal or oval although occasional elongate forms were seen.

The nucleus was vesicular and more constant morphologically than the cytoplasm. It was spherical, averaging 7 to 10 μ in diameter; occasionally it was ovoid or reniform. The chromatin clumps were coarse and rather sparse. Most cells contained one or two basophilic oval nucleoli. Occasionally three nucleoli were seen.

The cytoplasm was acidophilic, with a well marked granularity. A

narrow clear zone often was seen about the nucleus. Many cells were vacuolated but the degree of vacuolization was highly variable. Some cells had a zone of acidophilic granulation about the nucleus and vacuoles in the peripheral cytoplasm, an arrangement common in testicular Leydig cells. In one area the cells might be of smaller average size with a deep acidophilic and nonvacuolated cytoplasm and a darker nucleus, while adjacent areas might show larger cells with paler, more abundant cytoplasm and a clearer nucleus. The latter cells were more likely to show lipid vacuolization. These cell types may represent variations in the state of secretion of the cells.

Nelson[27] found giant multinucleated interstitial cells in 85 of 721 testes studied. Identical forms were found occasionally among the hilus cells. A group of these giant multinucleated ovarian hilus cells is illustrated in Figure 20.

Cytoplasmic Inclusions

Lipids. The cytoplasmic vacuoles contained lipids which stained brilliantly with the usual fat stains such as sudan III. As with testicular Leydig cells, the lipid content varied considerably from one cell to another. No stainable lipid was present in some cells; others might show only fine dust-like, sudanophilic droplets; and in some the cytoplasm was filled with large, irregular masses of sudanophilic material. Some, but not all, of the lipid droplets were anisotropic.

Pigment. Small round granules of golden-brown pigment were present in the cytoplasm of some cells, in both stained and unstained sections. The pigment granules took up sudan III to some extent. This pigment probably belongs to the group of lipochromes, and is identical in appearance with the lipochrome of Leydig cells. As in the testis, it is more prominent in older patients.

Crystalloids of Reinke. Testicular Leydig cells in human beings may contain characteristic "crystalloidal" cytoplasmic inclusions. These curious structures were first described by Reinke[28] in 1896 and, although nothing has been learned of their function, they are generally accepted as structures peculiar to Leydig cells. However, they are found also in the ovarian hilus cells, a fact which greatly strengthens the morphologic evidence of identity.

The crystalloids of Reinke are acidophilic rod-like structures within the cytoplasm. They are often as long as the greatest diameter of the cell, at times measuring 30 μ, more commonly averaging 10 to 20 μ. The ends are rounded or rectangular with blunted corners. Often a straight, clear line bisects the structure longitudinally. Commonly a clear zone or halo surrounds the structure. They are frequently multiple in

a given cell, tending to a parallel arrangement. Smaller, narrower types are found which have tapering or pointed rather than blunt ends. At times the crystalloids appear to be extruded partially from the cytoplasm of the cell.

The crystalloids were found in only a small percentage of hilus cells (as is the case with testicular Leydig cells) and patient search was necessary. In many ovaries, even with the study of a great many sections, they were not found. When present, they usually had a patchy distribution, abundant in localized areas and absent in adjacent clusters of hilus cells. When a single crystalloid was seen, others generally were found in neighboring cells. The significance of this distribution is not understood. Crystalloids in abundance and with the same patchy distribution were found in both of the cases of masculinizing tumors to be described.

What little histochemical data are available suggest that the crystalloids are protein bodies. They take the acid dyes strongly, are not sudanophilic, not doubly refractile, nor do they dissolve in the fat solvents. Eberth[29] stated that they swell in 10 per cent potassium hydroxide and dissolve in hydrochloric acid with pepsin, although they are insoluble in 10 per cent hydrochloric acid, nitric acid, and acetic acid.

I have observed spherical acidophilic bodies of varying size in the cytoplasm of cells in close proximity with crystalloid-containing cells. These "hyaline" spheres had the same staining reactions as the crystalloids, and may represent precursors of them. They were particularly prominent in the tumor cells in case 2 where crystalloids also were very abundant. In some fields the majority of cells which did not contain crystalloids contained these bodies. Often they occupied most of the cytoplasm of the cell, at times compressing the nucleus to one pole. Structures that appeared to be transitional between the spherical bodies and the crystalloids were present. These intermediate forms were ellipsoids, or broad rectangles with rounded corners. Some are illustrated in the camera lucida drawing (Fig. 23). These observations suggest that the typical crystalloids may develop by gradual transformation from pre-existing spherical cytoplasmic inclusions.

RELATIONSHIP TO NERVES

There was a constant relationship of ovarian hilus cells to the nonmyelinated nerves of the hilus. The cells were commonly found in masses irregularly ensheathing nerves. Even more striking was the presence of hilus cells, scattered singly or in groups, within a nerve trunk, lying between individual nonmyelinated nerve fibers and often producing localized bulging of the contour of the nerve. When nests were found

apparently isolated from nerves, serial sections almost always established contiguity with a nerve. I have confirmed the observations of Berger[5,8] and others that Leydig cells, located outside of the testis proper, have precisely the same relationship to nerve. In one instance, I also have observed a nonmyelinated nerve bundle within the testis proper, in which Leydig cells were dispersed in similar fashion within the nerve, separating adjacent nerve fibers.

RELATIONSHIP TO VESSELS

The ovarian hilus cells and their associated nerves often bear an intimate relationship to the vascular spaces of the ovarian hilus and mesovarium. The normal ovarian hilus has a remarkably complex vascular structure which has never been adequately explained. The bulk of the hilar tissue is made up of a tangled meshwork of small, tortuous, muscular arteries, large venous sinuses, and lymphatic spaces. Its vascular structure has recently been emphasized by the excellent injection preparations of Reynolds.[30] The number, size, and concentration of vessels seem far in excess of any reasonable vascular needs of so small an organ as the ovary.

Masses of hilus cells and nerve containing them were found in close association with large venous and lymphatic sinusoids. In fact, nerves containing or surrounded by hilus cells often formed distinct nodular protrusions into the lumina of vessels. In such areas the lumen appeared to be separated from the perineurium only by a layer of endothelial cells. Such structures are illustrated in Figures 7 and 8.

Hyperplasia of Hilus Cells Associated with Masculinization

Two instances of hyperplasia of ovarian hilus cells associated with clinical masculinization may be mentioned briefly. Both cases will be reported in greater detail in a subsequent study.

The first of these, Mrs. R. M., a 41-year-old para II, gravida II, had shown masculinization for 8 years. Among the pertinent findings were a heavy beard requiring daily shaving, a deep voice, large clitoris, and a masculine, although somewhat obese, habitus. Menses occurred about every 2 months.

The second case, Mrs. V. P., a 50-year-old para I, gravida II, gave a history of masculinization of 3 years' duration. Previously a well developed female, she grew a heavy beard, and her voice deepened. Menstrual periods, which had been regular, ceased 5 months before admission. Pertinent findings included obesity of abdomen, thighs, and hips, hirsutism of face and body, an enlarged clitoris, and atrophic breasts.

Both patients were subjected to abdominal exploration and panhysterectomy. The adrenals in both patients were normal grossly at operation.

Tissue taken for biopsy of an adrenal in the first case was not remarkable. In both patients the ovaries were eight to ten times the normal

size for the age, and were fairly firm. Except for a single small follicular cyst in the first case, no cysts or follicles were present. Microscopic examination revealed field after field of ovarian stroma with only several corpora albicantia interspersed. Of greatest interest, however, was the presence in both cases of a significant increase in the number of ovarian hilus cells.

Rare instances of greatly enlarged ovaries associated with masculinization are described in the literature.[31-34] No very satisfactory explanation of the masculinization in such cases has been proposed, although "hyperthecosis"[33,34] of the ovaries, and pituitary stimulation[32] have been advanced. It seems likely that the hyperplasia of ovarian hilus cells, in the 2 cases cited, was largely responsible for the development of masculinization. Whether this increase of hilus cells was secondary to other hormonal stimulation cannot very well be discussed within the scope of this paper. The significance of the great increase in bulk of the ovarian stroma is obscure. In this regard, however, the relatively large size of the ovaries in the case with bilateral hilus cell tumors, presented below, is worth emphasizing.

Hilus Cell Tumors with Masculinization

Berger,[8] in 1942, reported a case of masculinization associated with a small tumor of ovarian hilus cells. The patient, 50 years of age, had been masculinized for 18 years. There was facial hypertrichosis requiring shaving two or three times weekly, a masculine body build, deep voice with a prominent larynx, atrophy of the breasts, and an enlarged clitoris. In spite of these secondary sexual changes, she menstruated regularly. Following removal of ovaries, uterus, and tubes there was a significant regression of her masculinization. In the medial portion of one mesovarium there was a small tumor measuring 4.5 by 3.5 mm. composed of ovarian hilus cells. This is the only definite account of such a tumor which was found in the literature.

Another possible example of a similar neoplasm reported by Dreyfus and Barrozo do Amaral[34a] occurred in a female pseudo-hermaphrodite, but the picture was complicated by the simultaneous presence of aberrant corticoadrenal tumors in the region of the adrenal gland.

The following 2 examples of ovarian hilus cell tumors associated with clinical masculinization bring the total number of established cases to 3.* It is likely that others exist but have not been accurately diagnosed.

* Since this paper was submitted for publication, I have observed one additional case of hilus cell tumor, as well as 2 instances of hilus cell hyperplasia, all 3 associated with masculinizing syndromes.

Case 1

Clinical History. Mrs. C. G., a masculinized colored female, 86 years of age, was admitted to the Tulane Service of Charity Hospital on April 21, 1947, with a complaint of vaginal discharge and vague abdominal pain present for several months. Her childhood had not been remarkable and female characteristics had developed normally. She had borne three full-term children, all of whom had died in early infancy. Evidence of masculinization, as well as could be determined, dated from her 54th year, when she first began to grow a beard. Some time later her voice deepened. Because of her difficulty in recalling past events, little is known of her menstrual history, save that she had not menstruated nor had any vaginal bleeding for many years. No members of her family showed evidence of masculinization.

Physical Examination. Her features and habitus simulated those of an elderly male to a remarkable degree. She looked distinctly younger than her stated age and her skeletal musculature was remarkably well developed. She weighed 110 lbs.; her height was 136 cm. Her blood pressure varied between 140/88 and 164/100 mm. of Hg; pulse, between 58 and 70. She had a heavy mustache and a coarse, straggly beard. Her features were coarse and there was recession of the hairline at the forehead. The thyroid was not palpably enlarged. The thyroid cartilage was prominent and her voice deep. The breasts were small and atrophic and the nipples flat. A hard, nodular, nontender, movable mass was palpable in the lower abdomen, apparently arising within the pelvis and extending several centimeters above the symphysis. A male distribution of pubic hair was present; the labia majora and minora were atrophic. The clitoris was greatly enlarged, measuring 3.5 cm. in length by 1.5 cm. in diameter. The vaginal mucosa was not as atrophic as one might have predicted from the vulvar atrophy. The cervix was small and clean. Three or four stony masses projected from the sides of the uterus. The adnexae were not clearly outlined but it was felt that both ovaries were slightly enlarged and firm.

Laboratory Studies. Routine examinations of blood and urine were normal. Urinary concentration was good. The glucose tolerance test revealed a fasting level of 100 mg.; ½ hour, 135 mg.; 1 hour, 143 mg.; 2 hours, 103 mg.; 3 hours, 100 mg., per 100 cc. of blood. Twenty-four hour urinary 17-ketosteroids, as determined on three occasions by Dr. Albert Segaloff, were 6 mg., 9 mg., and 6 mg. The basal metabolic rate was —16.

Roentgenograms of the skull were normal, but those of the abdomen revealed multiple large areas of calcification in the lower abdomen and pelvis, compatible with calcified leiomyomas. Intravenous pyelograms were not remarkable.

The patient was considered a good surgical risk despite her age, and on May 1, 1947, was operated upon on the Gynecology Service. The adrenals were palpated at operation and were not enlarged. The ovaries, although of normal contour, were moderately enlarged for a woman of 86 years and the uterus was distorted by several partially calcified leiomyomas. A total hysterectomy and bilateral salpingo-oophorectomy were done. The postoperative course was uneventful save for a wound infection and she was discharged on June 20, 1947, in excellent health.

Pathologic Examination

The specimen consisted of an enlarged uterus with both tubes and ovaries attached. The cervical canal was stenotic for 2 mm. in the region of the external os. The uterus was grossly distorted by numerous leiomyomas, many of which were calcified. The endometrial cavity measured 6.5 cm. in length and 4 cm. in its greatest width. The endometrium was

pale pink, measuring less than 1 mm. in thickness. The tubes were not remarkable.

The ovaries were distinctly larger than would be expected from the advanced age of the patient. They corresponded roughly to ovaries of the 45 to 50 year age group, each measuring 3.5 by 2.5 by 2 cm. The serosal surfaces of both ovaries showed the usual pitting and corrugations. The ovarian tissue was firm but retained a slightly fleshy consistency. It was pinkish gray on section, with an indistinct tannish mottling. There were several corpora albicantia but no cystic structures present.

At the uterine pole of each ovarian hilus, lying within the mesovarium but contiguous with the ovarian stroma adjacent to the ovarian ligament, was a small, clearly demarcated tumor mass. The two tumors were remarkably symmetric in both position and size. They varied from a dark orange-brown to a dark olive-brown, and were of homogeneous fleshy consistency. The tumor at the left hilus measured 1 by 0.7 by 0.5 cm.; the tumor at the right hilus measured 1.2 by 0.8 by 0.5 cm.

Histologic Examination. The endometrium showed senile atrophy with cystic dilatation of the endometrial glands. The myometrium was atrophic. The leiomyomata showed considerable hyaline degeneration with extensive zones of calcification in some. There was chronic endocervicitis and dilatation of the endocervical glands. The tubes were not remarkable save for senile atrophic changes.

The ovaries did not show the advanced senile atrophy of the stroma expected in a woman of 86 years. The ovarian stroma was moderately cellular, comparable to that in the age group of 45 to 50 years. The tunica albuginea was of average thickness. A number of corpora albicantia were present. There were no follicular, thecal, or lutein cellular elements. Along the entire length of the ovarian hilus and adjacent mesovarium in both ovaries there was a striking abundance of hilus cell nests. The hilus cells showed the usual morphologic variations but were greatly increased in number. The usual relationship of hilus cell nests to nerves and vascular spaces was observed. Occasional crystalloids of Reinke were present within the cytoplasm of hilus cells. Figure 14 is a diagrammatic representation of the left ovary and adnexa as seen from behind.

Histology of Tumors. The bilateral tumors were composed exclusively of ovarian hilus cells, with a sparse accompanying stroma. They were not encapsulated. Occasional nonmyelinated nerve bundles lay adjacent to the periphery of the tumors and in some instances partially penetrated them. Neighboring hilus cells extended along the nerves so

that it was difficult in some regions to differentiate tumor cells from the hilus cells of the vicinity.

The two tumors were composed of well differentiated cells, many of which were indistinguishable from normal hilus cells. A considerable number, however, were larger, many measuring two and three times the diameter of average hilus cells. There was a greater variation in size and form in the tumor cells than in normal hilus cells. Many of the nuclei were irregularly shaped, but were otherwise comparable to the nuclei of hilus cells. Particularly toward the periphery of the tumors, the cells assumed elongate forms. A variable quantity of collagenous stroma was present in different parts of the tumor. Small groups of cells and often individual cells were surrounded by delicate collagenous fibrils. The cytoplasmic structures and staining reactions were identical with those of normal hilus cells.

Crystalloids of Reinke were abundant in the tumor cells, but the distribution was patchy, some areas containing numerous crystalloids, others being free of them. The crystalloids were mostly of the large variety, although occasional cells contained aggregates of small crystalloids. They were particularly well stained with the Masson trichrome stain and with phosphotungstic acid hematoxylin.

The cytoplasm was distinctly granular and acidophilic. Many cells showed marked granularity about the nucleus with vacuolization of the peripheral cytoplasm. With acid fuchsin, somewhat larger granules, 1 to 2 μ in diameter and lying within vacuoles, stained brightly. These were more prominent in the periphery of the cytoplasm. They correspond to the granules described by Whitehead[35] in his study of Leydig cells.

Mitochondria in the tumor cells were stained with phosphotungstic acid hematoxylin after preliminary mordanting of formalin-fixed material in 5 per cent aqueous ferric chloride after the method of Mallory. The mitochondria stained a deep blue. They were abundant in the form of round granules and short rods, duplicating the mitochondrial stains illustrated by von Winiwarter[36] in testicular Leydig cells. Some cells showed larger irregular clumps, small ring forms, and irregular networks in the peripheral cytoplasm. These probably represented mitochondria distorted by fixation as suggested by Duesberg[37] for the Leydig cells. They corresponded very closely to Duesberg's illustrations.

Many of the cells contained yellow-brown lipochrome pigment in small round granules in the cytoplasm, which probably accounted for the brownish color of the tumors. Sudan III stains of frozen sections revealed abundant but irregularly distributed lipid. Some cells were

free of sudanophilic material; the more vacuolated cells contained large amounts in the cytoplasm. Other cells contained only sparse small droplets of sudanophilic material. The lipochrome pigment took the stain to some extent. The variation in lipid content was comparable to that in Leydig cells. Some of the stainable lipid was doubly refractile when examined with the polarizing microscope.

Case 2

Clinical History. Mrs. E. W., a white woman, 64 years old, was admitted to the Tulane Medical Service of Charity Hospital on April 18, 1947. During the previous 2 years she had developed a beard and mustache, as well as increased hair on the arms and legs. Beard growth averaged ¼ inch weekly. During this period her voice had deepened. Two years previously she had been a normal female. She had borne two normal full-term children, now 35 and 33 years old. Her family history was not contributory. Her menstrual periods, which had been regular, ceased at the age of 40. There had been no subsequent vaginal bleeding except for an episode of spotting 8 months before admission.

Physical Examination. The patient was short, moderately obese, and weighed 146 lbs. There was congenital absence of the left arm below the elbow. The important findings included a marked facial hirsutism, and less marked hirsutism of the arms and legs. There was some recession of the hairline at the forehead. The pubic hair showed a tendency toward masculine distribution. The features were heavy and appeared masculine. The thyroid cartilage was slightly enlarged. Breasts and abdomen were obese. The blood pressure on different occasions varied between 110/60 and 146/96 mm. of Hg. On pelvic examination the clitoris was not grossly enlarged. A senile vaginitis was present, and there was a second degree cystocele. The uterus and adnexae were not definitely outlined.

Laboratory Findings. Results of routine examinations of blood and urine were within normal limits. A glucose tolerance test showed a fasting blood sugar of 103 mg.; ½ hour, 167 mg.; 1 hour, 121 mg.; 2 hours, 103 mg.; 3 hours, 105 mg., per 100 cc. of blood. Basal metabolic rates on three occasions were +16, +16, and +25. The Kline and Kolmer tests were negative. Roentgenograms of the skull and chest and retrograde pyelograms were not contributory. Determination of the 24-hour urinary 17-ketosteroids by Dr. Albert Segaloff showed 7.5 mg.

The patient was transferred to the Tulane Gynecology Service and a bilateral salpingo-oophorectomy and supracervical hysterectomy were performed on May 22, 1947. At operation the adrenals were not palpably enlarged. Her postoperative course was uneventful.

When seen 2 months after operation she complained of occasional hot flashes. Eight months after operation her voice had resumed a normal female pitch and there was a definite reduction in facial hirsutism.

Pathologic Examination

The uterus, removed supracervically, was of average size and contained no leiomyomas. The uterine cavity was small, with a thin endometrium. The tubes, except for slight thickening of the walls, were not remarkable.

The left ovary measured 2.8 by 1.3 by 1 cm.; the right ovary, 2.5 by 1.5 by 1.5 cm. Both were firm and grayish with the usual surface cor-

rugations. On section the ovarian stroma was grayish tan and free of cysts. In the inner medullary portion of the right ovary, midway between the poles, there was a spherical, yellow tumor nodule measuring 1 cm. in diameter. It was unencapsulated and fleshy. The tumor was surrounded by ovarian stroma for the most part, except for a portion which extended into the hilus region and adjacent mesovarium.

Histologic Examination. The endometrium was atrophic, with cystic dilatation of the endometrial glands. Moderate adenomyosis was present. The tubes showed healed chronic salpingitis.

The ovaries contained multiple corpora albicantia. The ovarian stroma showed moderate fibrosis and atrophy compatible with the age of the patient. The hilus cells in both right and left mesovaria were moderately increased in number although there was less hyperplasia than in case 1. Crystalloids of Reinke were fairly numerous. Nests of hilus cells lay in close proximity to the portion of tumor within the mesovarium. However, these were separated from the tumor, which was sharply circumscribed although not encapsulated.

Histology of Tumor. Sections of the tumor were prepared with hematoxylin and eosin, Masson trichrome, and sudan III stains. The histologic findings were very similar to those of case 1. The tumor was composed of well differentiated hilus cells, polyhedral and somewhat larger than average hilus cells. Mitotic figures were seen rarely. Few cells of the elongated form noted in case 1 were present, and the stroma was sparse and delicate. Nuclear and cytoplasmic details showed the same variations. Lipochrome pigment was present but less abundant. Stainable lipid was similar in amount and distribution to that in the tumors of case 1. Some of the lipid was doubly refractile.

Crystalloids of Reinke were present in even greater abundance than in case 1. Most of these were of the large type, some measuring as much as 35 μ in length. Occasional aggregates of smaller crystalloids were present also. As previously noted, numerous cells containing round acidophilic bodies with the same staining reactions as crystalloids were present. These may represent precursors of crystalloids. They were noted also in the hilus cells outside of the tumor and were more abundant than in case 1.

EVIDENCE OF FUNCTIONAL ACTIVITY OF OVARIAN HILUS CELLS

In these 4 patients with masculinization and with tumors or hyperplasia of hilus cells, the evidence is reasonably good that the hilus cells were functionally important. Do the ovarian hilus cells in the normal adult female produce androgens or do they merely represent vestigial structures? A precise answer is not possible in the present

state of our knowledge. The following facts, however, suggest that the cells normally have a secretory function.

The ovarian hilus cells are present at birth and can be identified during the first year or so of life. Following this and until the age of puberty they are absent or at least difficult to find. They reappear at puberty and persist through adult life, tending to decline in old age.[13] They are particularly prominent during pregnancy and at the menopause. These chronologic relationships suggest functional activity.

Furthermore, the cells have the histologic appearance of actively secreting cells. Cytoplasmic granules and lipid-containing vacuoles are prominent as they are in testicular Leydig cells. The histologic variations in the cells, as in testicular Leydig cells, suggest a life cycle of secretory activity. Crystalloids of Reinke in testicular Leydig cells do not appear until the age of puberty, when the cells become active. The only instance that I have seen of crystalloids of Reinke in the testes of a child occurred in a 4-year-old boy with precocious puberty of so-called constitutional or idiopathic type. Spermatogenesis was active and the Leydig cells were well developed, containing abundant crystalloids. Although the precise importance of the crystalloids is unknown, they appear to be associated (although inconstantly) with functional activity of the cells. The presence of crystalloids, then, in the hilus cells of normal adult women may reflect their secretory activity.

Finally, I should like to present evidence that the ovarian hilus cells respond to stimulation by chorionic gonadotropin. Ovaries from patients who had received 10,000 units of purified chorionic gonadotropin daily for at least 2 weeks were studied histologically.* Injections were begun shortly after the estimated time of ovulation. At this dosage, as reported by Brown and Bradbury,[38] menstruation may be delayed for 7 or more days. Significant changes were noted in the ovarian hilus cells in the 3 cases studied thus far. In all, the hilus cells were abundant and showed a severe though patchy degeneration (Fig. 10). In such areas the cytoplasm was dense and deeply acidophilic, cell boundaries were lost so that adjacent cells appeared to fuse together, and the nuclei were distorted and pyknotic, with a tendency for adjacent nuclei to clump together. Hilus cells in other areas were normal in appearance. A similar degenerative change in hilus cells was noted in the opposite ovary in a case of teratoma in which urinary gonadotropin was elevated. Brannon[21] reported similar degenerative changes in the hilus cells of a woman dying of pernicious vomiting of pregnancy. The possi-

* I am indebted to Drs. Brown and Bradbury for supplying me with ovarian tissue and adjacent mesovarium from one of their cases.

bility that the changes which he noted were due to chorionic gonadotropin stimulation must be considered.

Of particular interest was the presence of mitotic figures in the hilus cells of one case receiving large doses of chorionic gonadotropin. This case also showed degenerative changes in other groups of hilus cells. Mitotic figures are so rare in normal cells that none was found in over 100 routine specimens examined, although rare mitotic figures were present in the hilus cell tumors. Mitotic figures are equally rare in testicular Leydig cells.[39] The presence, then, of significant numbers of mitotic figures in the hilus cells of a patient who received chorionic gonadotropin is probably not fortuitous.

The evaluation of these morphologic changes in hilus cells with chorionic gonadotropin is not simple. However, as is well known, chorionic gonadotropin stimulates the growth and secretion of Leydig cells in the male. It is possible that the degenerative changes in the hilus cells are an exhaustion effect, particularly since the dosage of gonadotropin used was considerable. Nevertheless, the fact that hilus cells show responses to hormonal stimulation is additional evidence suggesting functional activity.

Discussion

Specific tumors of ovarian hilus cells are rare, as are ovarian masculinizing tumors in general. The case reported by Berger[8] and the two here presented are in fact the only established cases in the literature. It seems altogether likely, however, that some hilus cell tumors have been misdiagnosed as arrhenoblastomas, adrenal rest tumors, or luteomas, especially since the specific diagnosis of the masculinizing ovarian tumors is frequently a source of controversy among pathologists. Critical study of several reports of atypical masculinizing ovarian tumors strongly suggests that some of them may represent hilus cell tumors.

Clearly the hilus cell tumors should be separated from the broad and rather loose category of arrhenoblastomas. In the hilus cell tumors one finds neither the differentiated tubular structures nor the undifferentiated sarcoma-like pattern of the arrhenoblastoma. Unlike the more complex arrhenoblastoma, the hilus cell tumor represents a benign, well differentiated neoplasm composed of cells of a single type, which is similar to that of cells present in the normal ovarian hilus.

Although it is possible that tumors of hilus cells could be confused with adrenal rest tumors, there should be little difficulty in distinguishing adrenal rests of the ovarian hilus from hilus cell nests. The adrenal rests are usually yellow, spherical nodules, 1 to 3 mm. in diameter, sharply circumscribed and encapsulated. The hilus cells, in contrast, are

never encapsulated and tend to be dispersed in many small, irregular nests within the connective tissue of the hilus. Furthermore, the adrenal rests have an organoid structure, with cell cords centripetally arranged and duplicating normal adrenal cortex. Usually an indication of adrenal cortical zones is recognizable. The cells of the zona fasciculata are crowded with uniformly dispersed lipid-containing vacuoles, unlike the irregular and sparser lipid vacuoles of hilus cells. During the past year, we have seen in our laboratory at least six adrenal rests located in tissues adjacent to the adult ovary, as well as comparable structures adjacent to the testes in newborns. They are not likely to be confused with hilus cell nests. A typical example of an adrenal rest in an ovarian hilus is illustrated in Figure 25.

Certain aspects of the three known instances of masculinizing hilus cell tumors are worthy of comment, although generalizations are impossible on so small a group. In all, the tumors were of small size, 1 cm. or less in diameter. They were composed exclusively of ovarian hilus cells, well differentiated and apparently entirely benign. Crystalloids of Reinke, which, when present, are the most convincing identifying feature of these cells, were abundant in the tumors reported here. They were not present in Berger's case, nor need they be considered as a constant finding since they are present in only a fraction of normal hilus cells.

Excretion of urinary 17-ketosteroids in the two cases reported here were within normal limits, as may also be the case in women with masculinization due to arrhenoblastoma.[40,41] In contrast, 17-ketosteroids are more commonly elevated in masculinization due to adrenal lesions.

The persistence of menses in Berger's case and the lack of enlargement of the clitoris in case 2 indicate that the masculinizing syndrome need not be complete. The absence of hypertrophy of the clitoris in the latter case may reflect a difference in end-organ response to hormonal stimulation. Women treated with fairly large doses of testosterone for metastatic breast carcinoma show a striking difference in clitoris response, ranging from lack of detectable growth to marked hypertrophy.[42]

It is now well established that the normal adult female produces androgens. There is, in fact, but little difference either qualitatively or quantitatively in the urinary excretion of 17-ketosteroids in males and females.[43,44] The source of androgens in the female is generally considered to be the adrenal cortex. There is, however, considerable experimental evidence that the ovary, too, is capable of elaborating male sex hormones.[45-49] The specific cell producing these ovarian androgens is not clearly established although some investigators implicate lutein cells or lutein-like cells. It seems likely that in the human, one source of

androgen is the ovarian hilus cell, although its quantitative importance (compared, let us say, to the adrenal) is difficult to evaluate. The morphologic identity of the hilus cells with Leydig cells, their cytologic characteristics as secreting cells, their response to chorionic gonadotropin, and the evidence of masculinization with either tumors or hyperplasia of these cells all point to androgen production.

Further histochemical and physiologic investigations are needed to clarify the rôle of these cells in the normal female. The relationship of the hilus cells to sympathetic nerves and vascular spaces seems too striking to be fortuitous. That these anatomic relations may reflect some special physiologic mechanisms is an intriguing possibility worthy of further investigation.

Summary

Cells morphologically identical with testicular Leydig cells are regularly found in the human ovarian hilus and mesovarium. These ovarian hilus cells have a constant and intimate relationship to nonmyelinated nerves and vascular spaces. A similar relationship of Leydig cells to nerves and vessels is seen in the testicular hilus and adjacent structures.

Morphologic evidence, including the prominence of these cells at puberty, during pregnancy, and at the menopause, suggests functional activity. There is also evidence that they are responsive to stimulation by chorionic gonadotropin.

In the 2 cases of specific tumors of these cells and 2 cases of hyperplasia which are reported, there was masculinization.

The ovarian hilus cells may represent a further source of androgen in the normal female in addition to that derived from the adrenal cortex.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 71

FIG. 1. Two typical crystalloids of Reinke in the Leydig cells of the testis of a boy, 4 years old, with precocious puberty of idiopathic or constitutional type. Such crystalloids are a normal finding in mature testes. Crystalloids are seen in the lower left field; seminiferous tubules, above and lower right. (Slide was provided through the courtesy of Dr. Albert Segaloff.) Masson's trichrome stain. × 500.

FIG. 2. Ovarian hilus cells with abundant crystalloids of Reinke from a normal female, 39 years old, operated upon for uterine leiomyomas. Of note are the granular and vacuolated cytoplasm and acidophilic crystalloids. Hematoxylin and eosin stain. × 580.

FIG. 3. Ovarian hilus cells within and adjacent to a nonmyelinated nerve trunk of the mesovarium, a typical picture that may be seen in the hilus of normal ovaries. This is from the ovarian hilus at some distance from the hilus cell tumor in case 1. Hematoxylin and eosin stain. × 170.

FIG. 4. Hilus cell tumor in case 2, a 64-year-old masculinized female, showing large crystalloids of Reinke in tumor cells. Hematoxylin and eosin stain. × 670.

FIG. 5. Hilus cell tumor in case 1, an 86-year-old masculinized female, showing a variable distribution of sudanophilic substance in the cytoplasm of the cells. Frozen section: sudan III and hematoxylin stains. × 500.

FIG. 6. Hilus cell tumor in case 1, an 86-year-old masculinized female. Several crystalloids of Reinke are seen in one cell. The cells are larger than normal hilus cells, and the cytoplasm is abundant. A delicate collagenous fibril surrounds each cell. The brown pigment is lipochrome pigment. Masson's trichrome stain. × 450.

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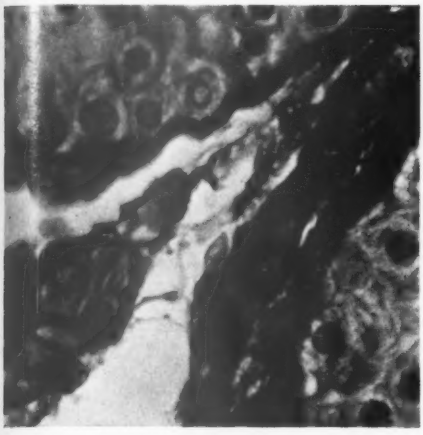

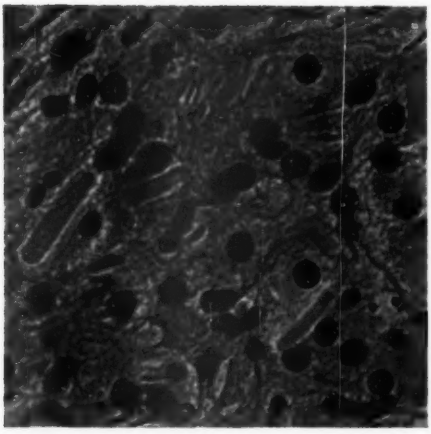

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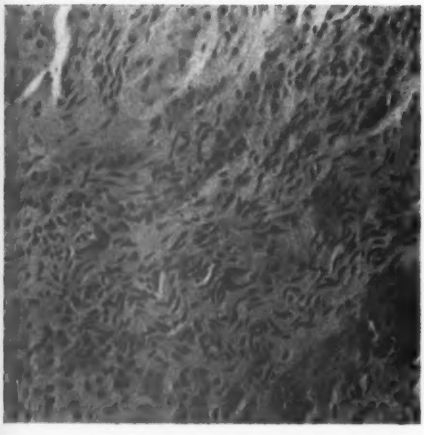

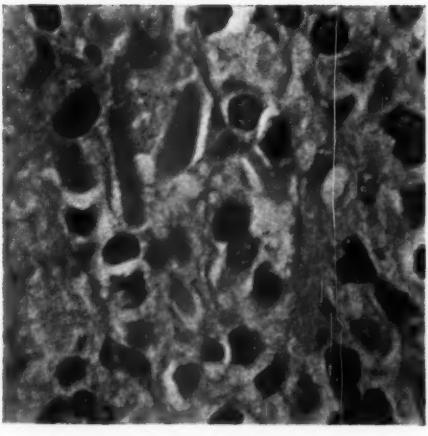

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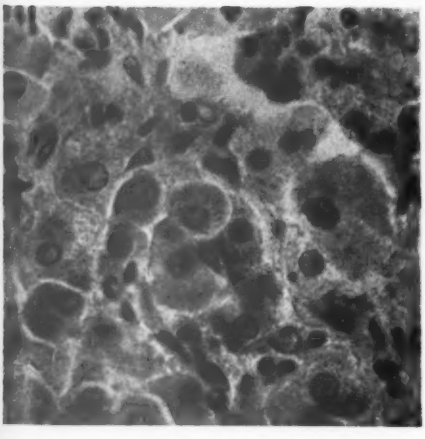

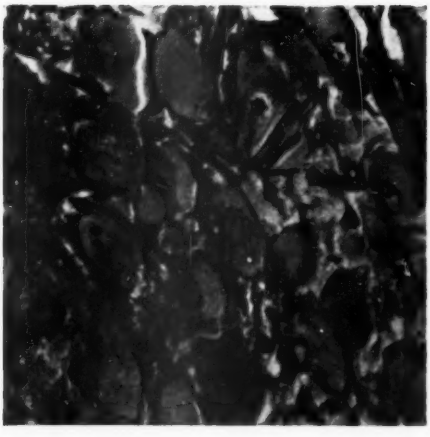

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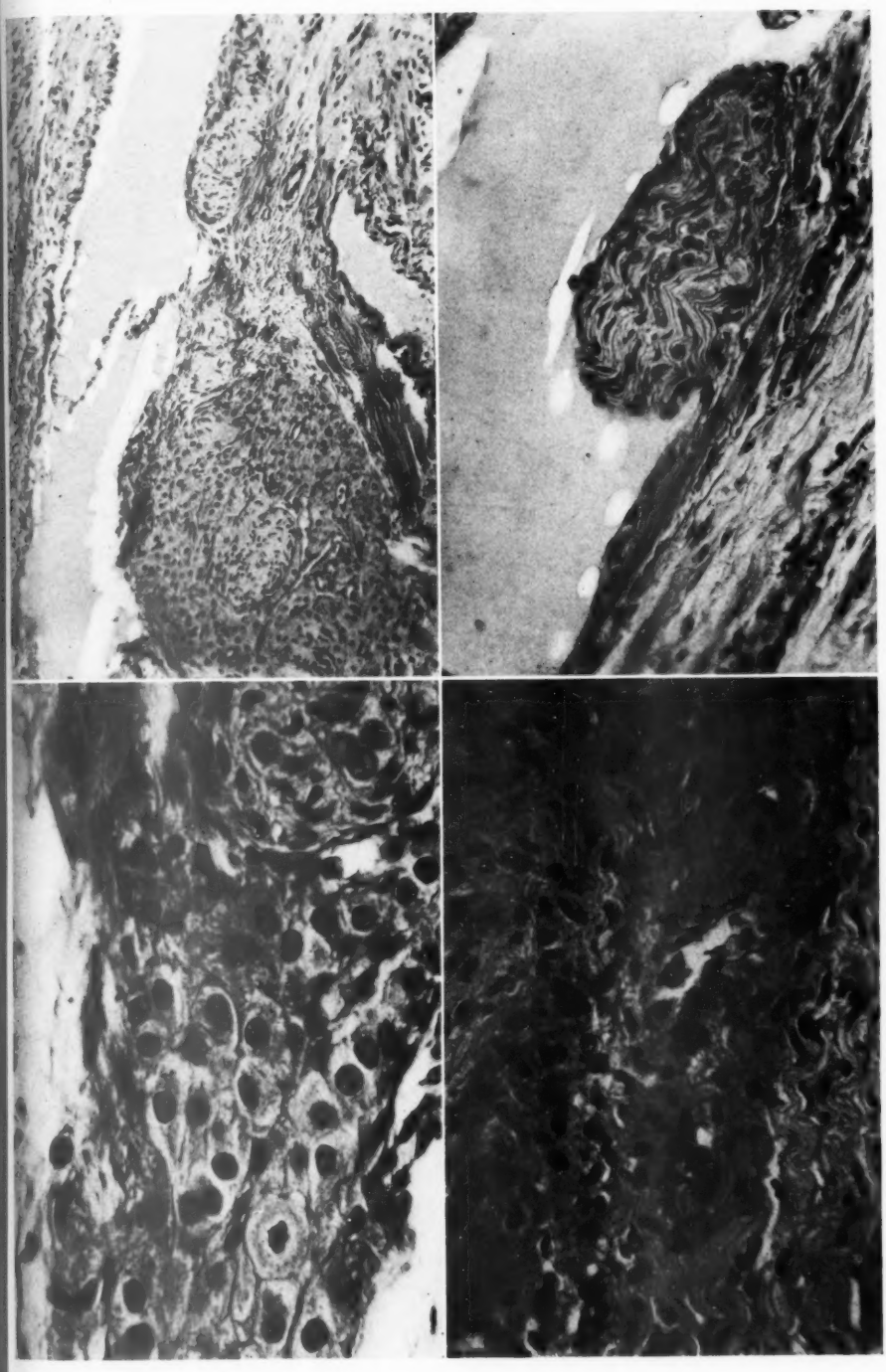
PLATE 72

FIG. 7. Nest of hilus cells and associated myelinated nerves protruding into a dilated lymphatic space in the mesovarium of a normal woman, 30 years old. Hematoxylin and eosin stain. × 120.

FIG. 8. Protrusion of nonmyelinated nerve and hilus cells into a dilated lymphatic space, from the same case as Figure 7. Hilus cells lie between nerve fibers. Only a layer of endothelium separates the structure from the lumen of the lymphatic. Hematoxylin and eosin stain. × 250.

FIG. 9. Hilus cells, with a mitotic figure in the lower part of the field, from a patient who received 10,000 I.U. of chorionic gonadotropin for 19 days. The nonmyelinated nerve bundle, upper right, contains hilus cells between nerve fibers. Hematoxylin and eosin stain. × 490.

FIG. 10. Hilus cells and nonmyelinated nerve in another portion of the mesovarium from the same patient as Figure 9. The hilus cells show degenerative changes, with dark, acidophilic, nonvacuolated cytoplasm, loss of cell borders, nuclear pyknosis, and clumping of nuclei. Masson's trichrome stain. × 390.



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Sternberg Ovarian Hilus Cells

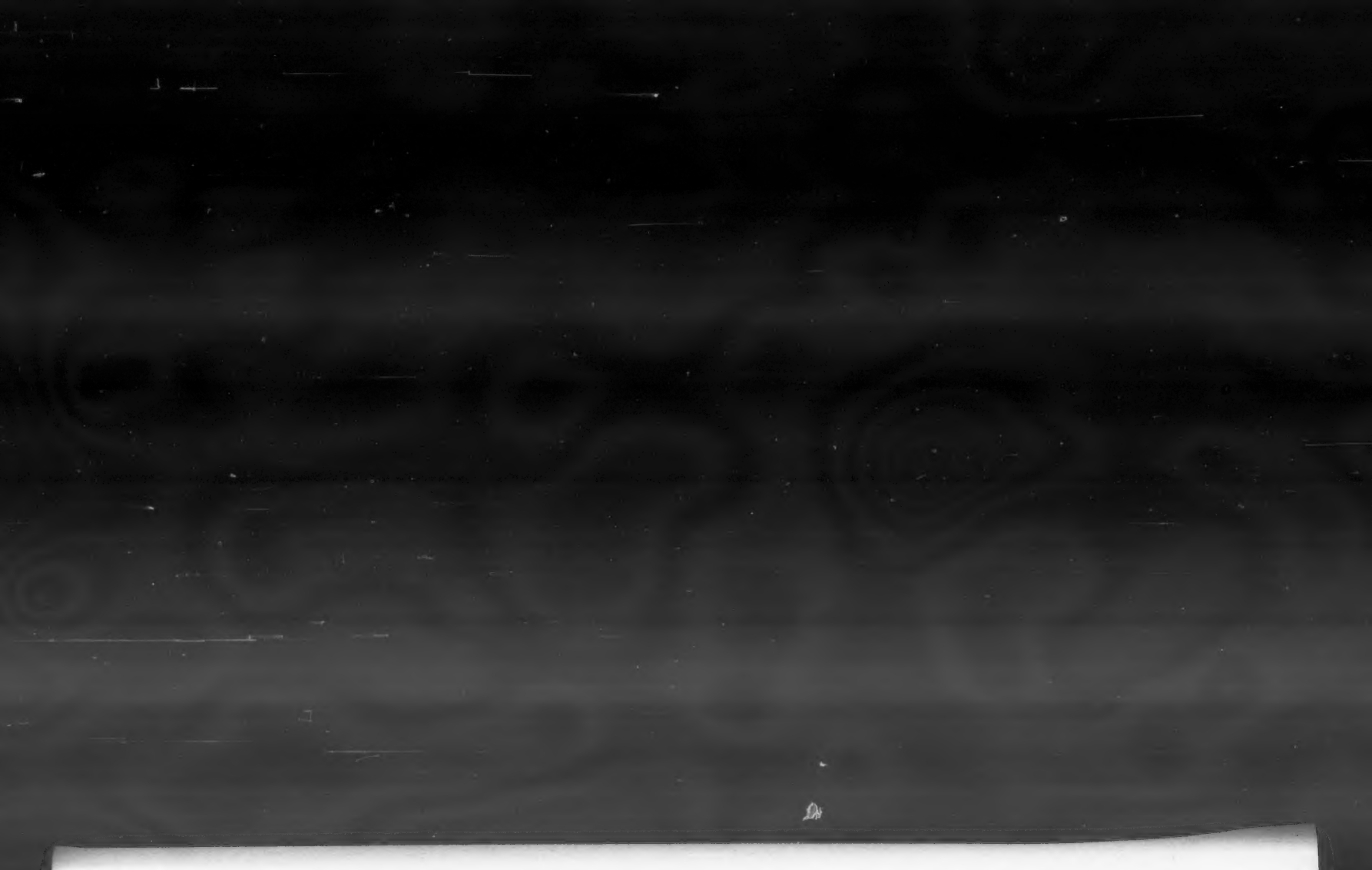
PLATE 73

FIG. 11. Photograph of patient, case 1, an 86-year-old colored masculinized female with bilateral ovarian hilus cell tumors. Of note are the masculine features, mustache, and beard growth. (Published with the written permission of the patient.)

FIG. 12. Photograph of enlarged clitoris, case 1.

FIG. 13. Photograph of ovaries with ovarian hilus tumors, case 1. The ovaries have been split longitudinally toward the hilus, and laid open before photographing. The dark areas in the upper portion of the left ovary and in the lower portion of the right ovary are the tumors. They were located symmetrically at the medial pole of each ovarian hilus.

FIG. 14. Diagram of left ovary, case 1, showing relationship of the tumor, ovarian hilus, and adjacent structures. The area within the dotted line is plotted by projection from actual microscopic sections and represents the ovarian hilus and adjacent mesovarium. The large black area represents the hilus cell tumor. The smaller black islands represent hyperplastic nests of hilus cells. The ovarian ligament is to the right of the tumor.



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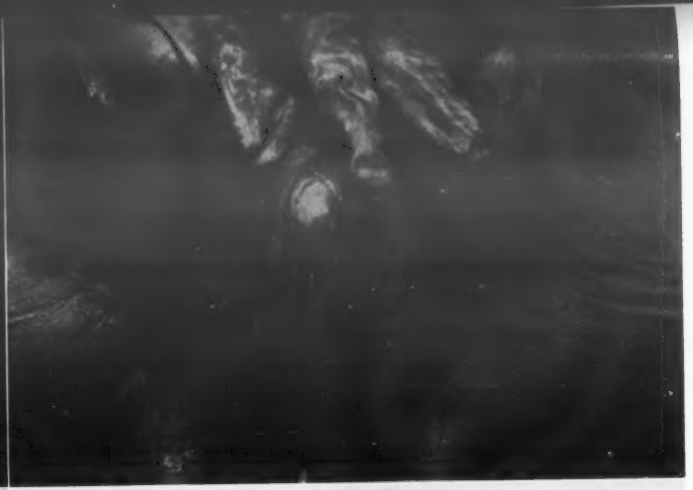

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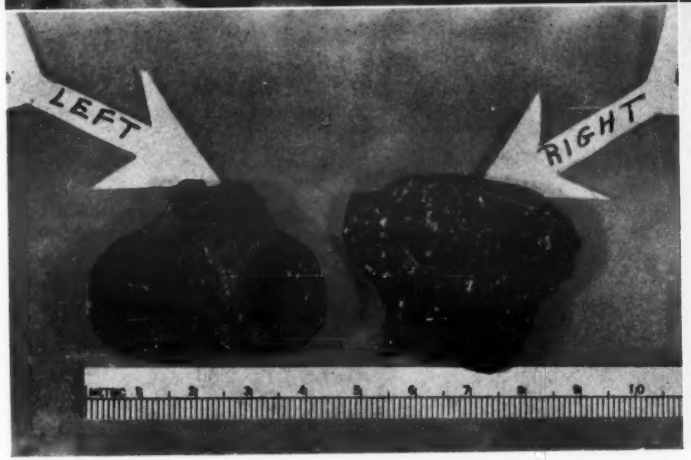

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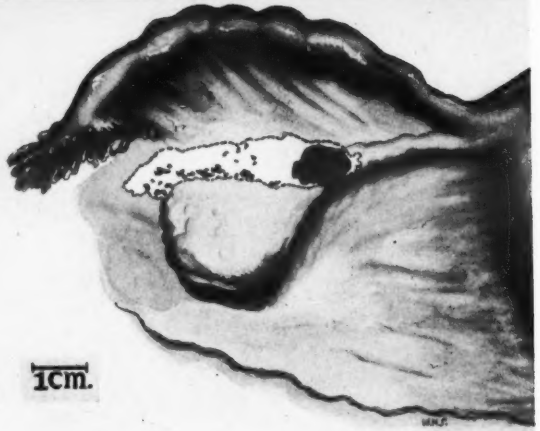


Ovarian Hilus Cells

PLATE 74

FIG. 15. Ovarian hilus cells and nonmyelinated nerve at the periphery of the hilus cell tumor, case 1. Hematoxylin and eosin stain. × 160.

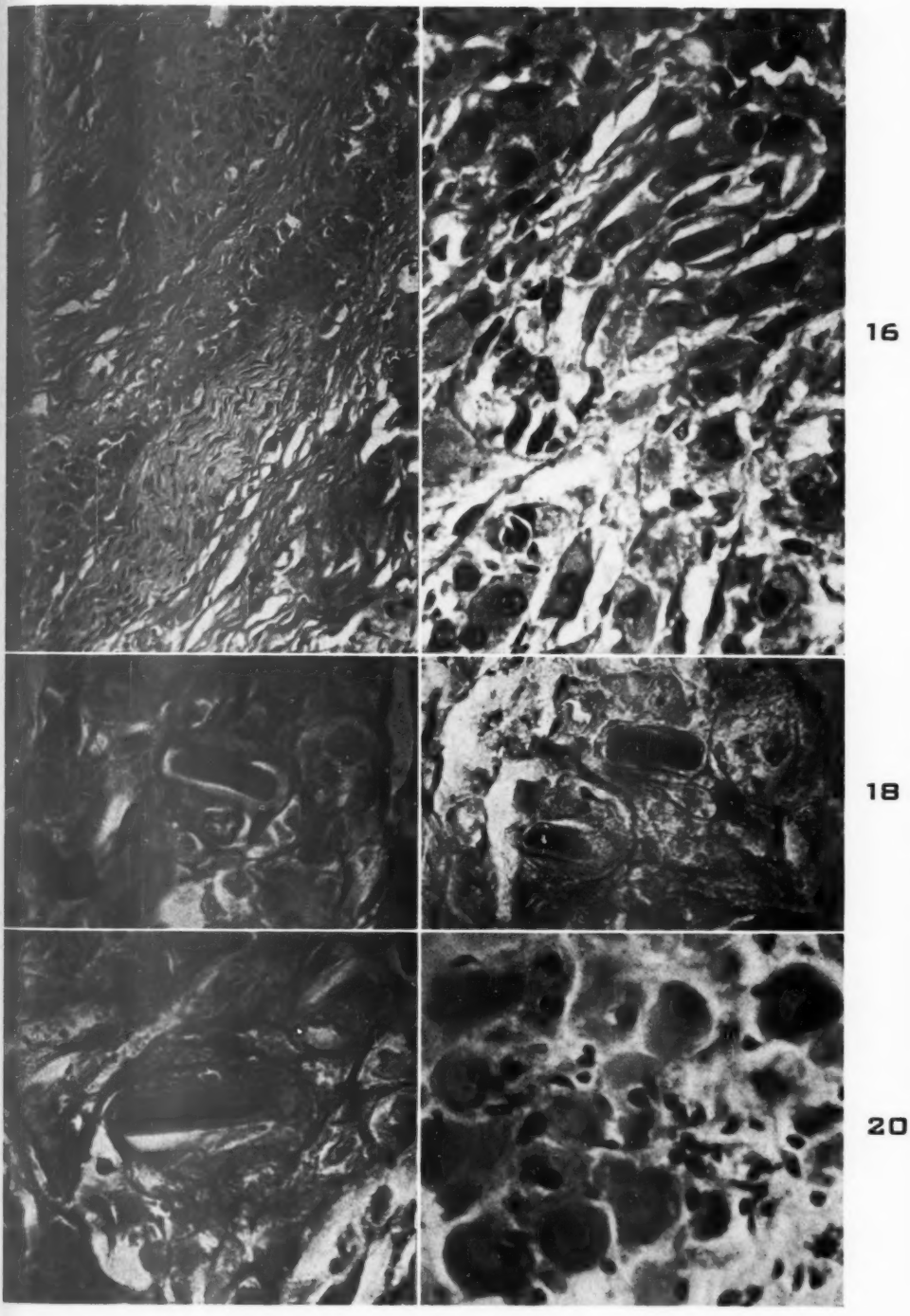
FIG. 16. Ovarian hilus cell tumor, case 1, showing elongated cell forms and greater morphologic variation than normal hilus cells. Crystalloid of Reinke is seen above and to the right of center. Two crystalloids are cut obliquely in the lower left field. Masson's trichrome stain. × 500.

FIG. 17. Crystalloid of Reinke from the hilus cell tumor, case 1. There is a clear space about the crystalloid. Phosphotungstic acid hematoxylin stain. × 800.

FIG. 18. Two crystalloids of Reinke from the hilus cell tumor, case 1. Delicate collagenous fibrils surround the cells. Phosphotungstic acid hematoxylin stain. × 650.

FIG. 19. Unusually large crystalloid of Reinke from the hilus cell tumor, case 1, with a surrounding clear space and longitudinal cleft. Phosphotungstic acid hematoxylin stain. × 800.

FIG. 20. Giant multinucleated hilus cells from the mesovarium of case 1, at some distance from the tumor. This is an occasional normal variation among hilus cells. Testicular Leydig cells may show similar forms. Hematoxylin and eosin stain. × 480.



Sternberg Ovarian Hilus Cells

PLATE 75

FIG. 21. Ovarian hilus cell tumor from case 2, a masculinized white female, 64 years old. The cells are very similar to normal hilus cells. Hematoxylin and eosin stain. × 160.

FIG. 22. Ovarian hilus cell tumor from case 2. Of note is the granular cytoplasm. Hematoxylin and eosin stain. × 670.

FIG. 23. Camera lucida drawing of tumor cells of case 2, showing "transition" of intracytoplasmic hyaline spherical bodies to crystalloids. Cells 1 and 2 (counting from upper left) contain ovoid and spherical bodies; cells 3 and 4 contain "transition" forms; cells 5 and 6 contain well developed crystalloids of Reinke.

FIG. 24. Photograph of masculinized 64-year-old white female, case 2, showing masculine features, recession of hairline, and mustache and beard growth. (Published with the written permission of the patient.)

FIG. 25. Typical adrenal rest in an ovarian hilus, an incidental finding in a routine surgical specimen from a patient 41 years old. Of note are the capsule, zone formation, organoid structure and simulation of normal cortex. For comparison with hilus cell nests. Hematoxylin and eosin stain. × 120.

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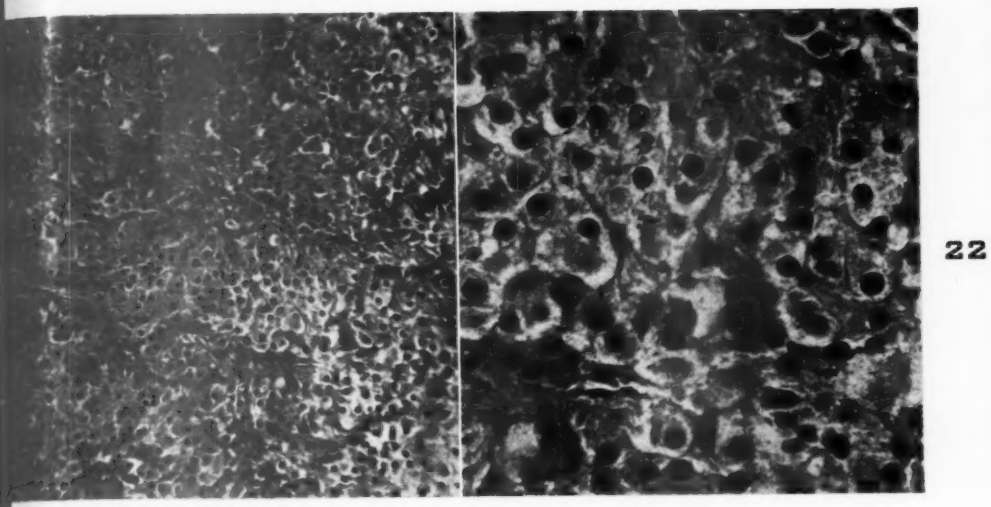

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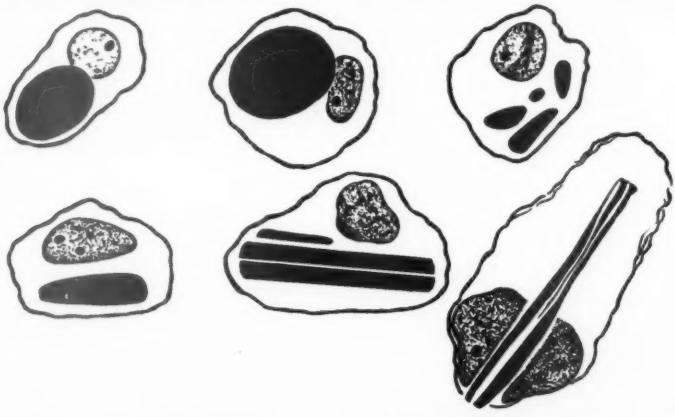

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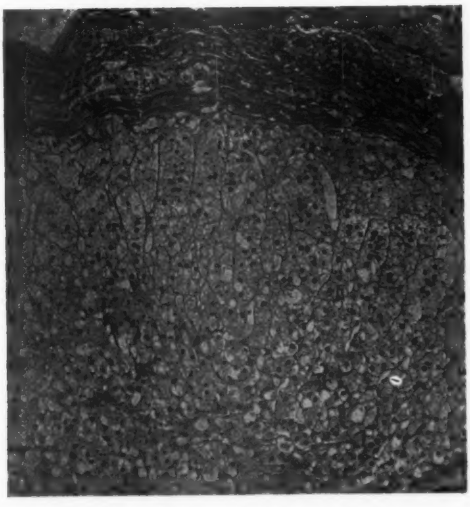

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Ovarian Hilus Cells

OXYPHILIC GRANULAR CELL ADENOMA OF THE PAROTID GLAND (ONCOCYTOMA)

Report of Five Cases and Study of Oxyphilic Granular Cells (Oncocytes) in Normal Parotid Glands *

Luis Meza-Chávez, M.D.†

(From the Departments of Pathology of Memorial Hospital, New York, N.Y., and of the University of Michigan, Ann Arbor, Mich.)

Although the parotid gland is the most common site of neoplasms of the salivary glands,[1-5] a pure adenoma in this location is considered a rarity.[2-11] McFarland[9] reported a "possible case," reviewed the literature, and concluded that "nearly all, if not, indeed, all, of the described adenomas are but mixed tumors of unusual appearance." This statement is now no longer valid since the literature contains several reports of true adenomas of which McFarland's case is a good example. A study of the reported adenomas leads to the impression that some of them belong to a definite neoplastic entity, because of the similarity of their cellular components. The characteristic cells have been variously designated. They are epithelial in origin and larger than the normal cells of the parotid gland; they have granular or reticulated oxyphilic cytoplasm, according to the stain employed. Their nuclei are dark-staining, frequently pyknotic, and with irregular contours.

Schaffer[12] was the first to describe these cells as "granular swollen cells." He found them in the ducts and acini of salivary glands of the tongue, uvula, pharynx, and esophagus. Pischinger[13] described them in the sublingual gland. Zimmermann[14] observed them in the trachea, floor of the mouth, and sublingual glands. Because of the characteristics of their nuclei he called them pyknocytes. Hamperl[15] studied the uvular, sublingual, and submaxillary glands of 85 persons whose ages ranged from infancy to 96 years. He gave a very complete and detailed description of the same cells and called them "onkocytes" because of their increased size. He found them occasionally in persons under the age of 50, but they occurred more frequently between 50 and 70, and were present practically always in those over 70 years of age. These peculiar cells sometimes formed tumor-like areas of hyperplasia disposed as solid or adenomatous foci.[15,16] In addition he described transitional forms between both ductal and acinar cells and the "onkocytes."[15] Steinhardt[11] examined the sublingual, submaxillary, and parotid glands of 65 persons and found 8 examples of what he thought were the "transitional form"

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† Hubert E. Rogers' Fellow in Pathology.

between normal cells and the "onkocytes" described by Hamperl[15]; these "transitional forms" were found in 5 persons over 80 years of age and in 3 between 60 and 80 years. Steinhardt failed to mention specifically in which of the glands he found the cells. He also observed nodular hyperplasia in the submaxillary glands of 2 of his cases; one of these was of the solid and the other of the adenomatous papillary type. Hamperl[17] wrote that the cells of the "transitional form" of Steinhardt were "onkocytes." Škorpil[10] mentioned that in 100 salivary glands he found 5 examples of "oncocytes" forming nodular hyperplasia. The areas of hyperplasia were present in submaxillary, sublingual, and nasal mucosal glands. Stout[18] found cells of the same type in the bronchi of adults and discussed their possible relationship to the cells of bronchial adenomas. Nohteri[19] studied the mucous membrane of the nose and of the larynx of 41 and 37 persons, respectively, and his results were similar to those of Hamperl.[15] He found "oncocytes" only in persons over 52 years of age and regularly in those over 70. In addition, he described a cyst in the larynx composed of "oncocytes."

"Oncocytes" have been found also in other organs. Hamperl[20] listed the following: Parotid, submaxillary, sublingual, and minor salivary glands; thyroid gland, parathyroid gland, hypophysis (anterior and posterior lobes), testicle, fallopian tube, pancreas, liver, stomach, and the glands of the pharynx, trachea, and esophagus. Hamperl[17] also mentioned that Veratti[21] had noted similar cells in the salivary glands of dogs.

McFarland[9] reported what seems to be the first proved case of pure adenoma of the parotid gland composed of the oxyphilic granular cells.* At that time he was not convinced that the lesion was a true adenoma, but seemed inclined to consider it as a mixed tumor of unusual appearance as shown by his conclusion: "The occurrence of gland-like tumors (adenomas) and of tumors containing varying quantities of glandular tissue is no indication that such tumors arise from the glandular tissue proper, but simply goes to show that glandular tissue may sometimes preponderate over other tissue components of mixed tumors." This view was restated in 1936[22] when, on commenting about the recurrence of the tumor after 12 years, he said: "Subsequent events fully justified its original classification as a mixed tumor."[23] However, in 1942[24] and 1943[25] he referred to this adenoma as a "Hürthle cell tumor (onchocytoma)" and placed it under the group of "tumors mistaken for mixed tumors."

* The term "oxyphilic granular cell" will be used as a synonym of "oncocyte" and of other terms used to designate the cells under discussion.

Gruenfeld and Jorstad[26] reported the second example. They were the first to point out the similarity of the "onkocytes" of Hamperl to the cells of the case reported by McFarland as well as to those of their own. Their case appeared to be of multicentric origin. They expressed the opinion that it had originated from the duct system, and they called their lesion an "onkocyte tumor." Harris[27] reported a case which seemed to be of multicentric origin from the ducts. He objected to the name onkocytoma and stated: "Inasmuch as the onkocyte has also been named pyknocyte, this tumor could be called pyknocytoma, but the need for any such name is not apparent." Škorpil[10] reported 4 cases of adenoma of which the fourth was another example of parotid tumor composed of "onkocytes." As did the two previous authors, Skorpil stated that the tumor arose from the ducts, and added that the name "onkocitäres" adenoma or "onkocytoma" would be appropriate for such a tumor. Ackerman[6] recorded another example of adenoma and tabulated the reported cases up to that date. He reported his case under the name "oncocytoma of the parotid gland." Lloyd[8] reported the most recent case of parotid adenoma of the type under review, which is case 8 of his paper. He used the name "oncocytoma of the parotid" to designate his tumor. The only other proved case of tumor of this type found in the salivary glands is that of Ahlbom[1] who reported an adenoma of the hard palate which probably arose from the minor salivary glands of that region.

Besides these cases which can definitely be accepted as examples of the type of tumor composed of oxyphilic granular cells, there are some others which may be considered as possibly belonging to the same group, and also a few which have been included by some authors without sufficient justification. One probable case is the first of the two adenomas reported by Hückel[28] of which part of the description and one illustration (his Fig. 1) have some resemblance to the tumors under discussion. Another is one of the two parotid adenomas briefly described by Blair and Olch.[3] The possible acceptance of one of these is based on the fact that Gruenfeld and Jorstad,[26] who had an opportunity of examining the cases of Blair and Olch, stated that "one of the two specimens was found to be an exact replica" of the tumor they reported. McFarland[24,25] mentioned another case that probably is a tumor of this type. He included it, together with his case reported in 1927, under the title "Hürthle cell tumor (onchocytoma)." However, he mentioned only that the patient was a female, 74 years old, who had had a tumor in the left parotid gland for many years. The tumor was excised, but he did not describe or illustrate it. Another possible case, because of the description given, is the one

reported by Duplay[29] which was examined by M. Ravier, but was not illustrated. Of the cases included in the group without adequate justification are two adenomas reported by Stöhr and Risak,[30] another reported by Franssen,[31] and one more reported by Steinhardt.[11] The cases of Stöhr and Risak should not be included for the reasons already mentioned by Harris[27]; Franssen's case, about which Lloyd[8] said "there seems no good reason for not including this tumor among the oncocytomas," cannot be accepted because neither the description nor the illustrations is characteristic of the tumors here discussed. Steinhardt's case cannot be included because I believe that this case represents a different type, of which I have had an opportunity to study a few examples that proved to be unlike the ones described in this communication.

Tumors formed by "onkocytes" have been reported in some other organs besides the salivary gland. Hamperl[20] summarized those cases and listed the pancreas, thyroid and parathyroid glands, and hypophysis as sites in which they have been described.

Oxyphilic Granular Cells in Normal Parotid Glands

From the review of the available literature, it is evident that despite the statement[20] that "onkocytes" have been found in the parotid gland, no definite proof has been given of their demonstration in normal parotid glands. This made apparent the necessity of finding out if they are present in the parotid gland and if they form foci of nodular hyperplasia similar to those described in the other salivary glands. Their demonstration was considered especially important since, with one exception, all of the salivary gland tumors composed of "onkocytes" occurred in the parotid. It is the purpose of this communication to summarize the results of the search for "onkocytes" in 100 parotid glands and to report 5 new cases of parotid adenoma believed to correspond to the group here reviewed.

The 100 parotid glands were obtained from 51 persons on whom autopsy was performed in the University Hospital (Michigan). These were unselected persons who died from various diseases or from accident or violence. Their ages ranged from 7 months to 76 years. Twenty-nine were under 50 years of age, 17 between 50 and 70 years, and 5 were over 70 years.

Most of the material was fixed in 10 per cent formalin, but absolute alcohol also was used in some cases. The sections were stained routinely with hematoxylin and eosin, and Mayer's mucicarmine stain was done in every case in which oxyphilic granular cells were found. Best's carmine stain was used also in the few instances in which the alcohol-fixed material showed the cells under study.

Oxyphilic granular cells were found in 9 of the 51 persons from whom material was studied (Table I). They were present in both parotid glands in 8, and from the ninth only one parotid gland was obtained. The cells were found in ducts, acini, or both, and they were usually seen in several areas of the sections studied from each case. Some of the ducts lined by oxyphilic granular cells were dilated and contained oxyphilic granular material (Figs. 1 to 4); others showed infolding of their walls (Fig. 3). In 4 of the 9 persons the oxyphilic granular cells

TABLE I

Cases in Which Oxyphilic Granular Cells Were Found in the Parotid Glands after Necropsy

No.	Sex	Age	Cause of death	State of the parotid glands	Remarks about oxyphilic granular cells
1	M	68	Carcinoma of the stomach	Slight atrophy	Lining ducts
2	F	74	Carcinoma of the breast	Slight atrophy	Transition of duct epithelium into oxyphilic granular cells; lining ducts, and showing adenomatous hyperplasia
3	F	67	Chronic lymphatic leukemia	Moderate atrophy	Lining ducts
4	M	49	Chronic myelogenous leukemia	Marked atrophy	Lining ducts
5	F	64	Third degree burns (carcinoma of the thyroid)	Slight atrophy	In acini
6	M	60	Lobar pneumonia	Marked atrophy	In ducts and acini, with adenomatous hyperplasia
7	F	70	Carcinoma of the thyroid	Marked atrophy	In ducts and acini, forming adenomatous hyperplasia
8	F	75	Carcinoma of the stomach	Moderate atrophy	In acini
9*	M	50	Bullet wounds	Normal	In ducts and acini, with adenomatous hyperplasia

* Only one parotid gland was obtained.

were forming areas of "hyperplasia" of the adenomatous type (Figs. 1 and 2). Sometimes two or three areas of "hyperplasia" were seen in different fields in one section.

In order to avoid unnecessary repetition, the detailed description of the oxyphilic granular cells will be given in the microscopic description of the adenoma of case 1, but it should be stated that both Mayer's mucicarmine and Best's carmine stains failed to reveal mucin or glycogen in the oxyphilic granular cells. From Table I it can be seen that the youngest person in whom oxyphilic granular cells were found was 49 years old and the oldest 75 years; neither sex was preponderant, and granular atrophy was not particularly associated with the occurrence of such cells except in the youngest person in whom there was a marked atrophy. Areas of "hyperplasia" formed by oxyphilic granular cells were more frequent in this series than in the one of Steinhardt,[11] in which only 2 examples of hyperplasia were found in the salivary glands

of 65 persons. The list of causes of death shows that with two exceptions oxyphilic granular cells were present in persons with a neoplastic process. This relationship may not be significant since the cells occur in a period of life when neoplasms are commonly found. Also, the necropsy service from which this material was obtained is heavily weighted with cases of neoplastic disease. A study of a larger and more representative series is necessary to evaluate this relationship, which was not brought out in Hamperl's study.[15]

Report of Cases of Adenoma

Case 1

The patient (Memorial Hospital accession no. V9578) was a 50-year-old male who gave the following history at the time of admission: Two and one-half years previously he had noted a small area of fullness in the upper part of his neck just below the lobe of the left ear. This area did not appear to grow until 6 to 8 months before admission, after which it slowly increased in size. During the last 3 months the swelling had extended to the pre-auricular and post-auricular regions. There had been no pain or discomfort. One month prior to admission, a small pea-sized nodule appeared in the post-auricular fold. It was tender, red, and spontaneously drained sanguineous fluid about 2 weeks later. After that it decreased markedly in size and tenderness.

On examination there was a soft, somewhat rubbery mass, 4.5 cm. in diameter, in the region of the tail of the parotid gland, just below the lobe of the left ear. The tumor was nontender and nonfluctuant. The posterior margin of the mass was in the post-auricular region. Immediately superior to the mass in the skin of the post-auricular fold there was a 6 mm. reddish nodule which had the gross characteristics of a recently infected "sebaceous cyst." The superior portion of the parotid gland was normal to palpation. Pressure over the mass caused clear saliva to drain from the orifice of Stensen's duct. There was no regional adenopathy or notable intra-oral lesion. The tumor was aspirated and the report upon the material was "salivary gland and lymphocytes." The possibility of Mikulicz' disease was considered and a small amount of radiation was given, which caused no change in the tumor. The material obtained from a second aspiration showed "salivary gland tissue only." Because of the persistence of a well encapsulated, freely movable tumor, the patient was admitted to the hospital for surgical treatment. The surgeon found a lobulated, well encapsulated, pinkish tumor about 3.5 cm. in diameter in the tail of the parotid gland; grossly, the tumor looked like three matted hyperplastic nodes. The patient made an uneventful recovery, with no evidence of recurrence 16 months after operation.

The tissue removed surgically consisted of several nodules resembling lymph nodes in fatty tissue. The largest measured 3.5 by 1 by 1.5 cm.; another was 1.5 by 1 by 1 cm., and four small ones had an average diameter of 6 mm. They were soft, fleshy, and brownish gray on section.

Sections from each of the nodules examined showed a similar appearance. The nodules were surrounded by an incomplete, thin, connective tissue capsule, outside of which there was a small amount of normal parotid gland, which was present also at an area where the capsule was absent. The tumor was composed of lobes separated by connective tissue.

The lobes were divided into irregular lobules by thin fibrous strands. There was some fatty tissue, particularly about the periphery of the tumor and within the normal parotid gland. Blood vessels of medium size were seen throughout. The lobules were formed by masses of epithelial cells arranged in a solid fashion, forming cords composed of single or double rows of cells (Figs. 6 and 11) or adopting a tubular or acinar pattern (Fig. 10). The epithelial cells lay on a thin basement membrane which in some cases was indistinguishable from the walls of capillary blood vessels running between the cords of cells (Fig. 6). The cells were large, spherical, oval, columnar, pyramidal, or polyhedral depending on the pattern which they formed. Their boundaries were clearly seen when they formed cords, acini, or tubules, but were difficult to make out in the solid masses. The cytoplasm was abundant, granular, and stained intensely with eosin (Fig. 6). The granules were small, regular in size, and stained reddish with Masson's trichrome stain and purplish blue with Mallory's aniline blue. A fine reticulated structure could be made out in some of the cells in which the granules were in the spaces of the network. The nuclei were small, round or oval, with fine granular chromatin evenly distributed. They had a prominent nucleolus, and sometimes two nucleoli were seen. Some of the cells had two nuclei or an elongated nucleus with a constricted area in its middle portion. Throughout the sections there were some cells which stained more intensely. They were elongated, pyramidal, or irregular, with dark, irregularly indented nuclei in which no nucleoli could be seen. Their more eosinophilic cytoplasm contained slightly larger granules. These cells often were in pairs or in small groups.

The ducts were markedly reduced in number and only a few could be considered normal. Some of the excretory ducts were distended with coarsely granular substance in which a few leukocytes and desquamated cells were seen (Fig. 7). A few ducts showed flattened epithelium which progressively changed into a cuboidal type with the characteristic oxyphilic cells. When studied in serial sections, one branched duct with the features previously described showed the cells of its ramifications to be continuous with cords of tumor cells (Fig. 7). In some instances the striated ducts, lined with eosinophilic cells, were seen also to form a unit with cords of tumor cells, all of them resting on a continuous basal membrane.

Some of the intercalated ducts were lined by stratified squamous epithelium and ended in glandular structures. These glandular formations had a basal membrane on which rested a row of low-cuboidal or flattened epithelial cells; toward the center the cells increased in size and became vacuolated. These cells had central nuclei; the cells closer to

the lumina of the ducts had irregular pyknotic nuclei. They were seen also in the lumina of some of the ducts as if forming a holocrine secretion. The structures were similar in all respects to sebaceous glands. These glands were observed also to arise from striated ducts in a few instances.

There was an area of parotid tissue in one section, between two lobules of tumor, in which, in addition to the normal acini with cells containing bluish or purplish blue granular cytoplasm, there were other acini, the cells of which stained from light pink to a marked eosin red. Such acini seemed to represent different stages of transformation from normal acini into those of the fully developed adenomatous type. The acini which showed the earliest change were of normal size but the granules of their cells were eosinophilic. A narrow, clear halo could be seen around each nucleus (Fig. 8). In a more advanced stage, cells and acini were larger than is normal. The cells had a more eosinophilic granular cytoplasm and the clear halos around the nuclei were still present in some of the cells (Fig. 9). When the changes were more advanced, the acini were formed by cells with all of the characteristics of those in the tumor, except that they were slightly smaller (Fig. 10). In this stage, a few of the cells showed a very fine, faint, perinuclear halo. In all of these stages the nuclei showed no noticeable change. The different features described in the acini were seen to occur also in the intercalated ducts, which in some cases contained a granular eosinophilic substance in their lumina.

Throughout the sections there were lymphocytes forming irregular groups but without follicle formation. They were frequently seen around ducts and they were more numerous in these areas (Fig. 7). There was no evidence of the presence of any of the components of the "mixed tumors." Careful search of the sections failed to reveal cells undergoing mitosis.

Case 2

The patient was a female, 72 years old, who was admitted to the University Hospital with the chief complaint of a swelling in the right side of the neck, present for 7 to 10 years. When discovered it was the size of a small walnut, but it had increased during the last 4 to 5 years. There were no other symptoms.

On examination there was an elongated tumor, soft and uniform in consistency. It extended from the right mastoid and parotid regions downward for a distance of about 6 cm. The tumor was movable, nontender, and measured about 4 cm. in width. It was removed surgically and the surgeon stated that it was well encapsulated, lobulated, and soft. It extended from the level of the hyoid bone to a point 2 cm. above and behind the angle of the jaw. The tumor was easily stripped from its capsule after several large vessels passing through it had been clamped and divided. There was mild facial palsy after the operation. The patient recovered uneventfully and her palsy completely disappeared. She was without evidence of recurrence 12 years and 8 months after operation.

The tissue removed surgically (University of Michigan, no. 1406-AN) was a flat, lobulated tumor measuring 8 by 5 by 2.5 cm. with a uniformly firm consistency. On section it showed a dark brown, lobulated surface.

Microscopic sections revealed a lobulated tumor surrounded by a thin fibrous capsule. The lobes were separated by septa which in some areas resembled those of a normal parotid gland. A small amount of non-neoplastic parotid gland was seen outside the capsule and also inside of it about the periphery of the tumor (Fig. 14). The parotid tissue just inside the capsule was greatly compressed. There were many thin-walled blood vessels in all sections. This tumor was similar in appearance to that of case 1, with areas of solid and loose structure. There was fatty tissue throughout the section, but it was more abundant in the less solid areas. Normal ducts were absent from all of the sections. The few ducts present were seen in the interlobar septa. They were lined by stratified squamous epithelium and one of them contained desquamated epithelium in its lumen. There was lymphocytic infiltration around some of the ducts; there were groups of lymphocytes in other areas of the tumor also, but they never formed germinal centers. The tumor cells, like those in case 1, were arranged in cords, acini, tubules, and gland formation (Figs. 12 and 13). The cellular details were similar to those of the previous case except for areas in which the granular appearance of the cytoplasm was not so apparent. The granularity of the cytoplasm was best observed in sections stained with Mallory's aniline blue. The nuclei had the same characteristics as in case 1, although pyknotic nuclei were not present in cells with the more eosinophilic cytoplasm, a type of cell rarely seen in this case. Some cells were binucleate. At the periphery of the tumor there was an area of dilated ducts lined by cells of the same type as those seen in the tumor. Some of the ducts contained an eosinophilic granular material (Fig. 14). The same substance occasionally was seen in the lumina of duct-like structures throughout the tumor. The normal parotid tissue showed areas in which the acinar cells were slightly eosinophilic, suggesting the earliest state of transformation into tumor, as described in case 1. Study of numerous sections failed to show any myxomatous, cartilaginous, or other tissues usually seen in the so-called "mixed tumors" of the parotid gland. Mitotic figures were not seen in any section.

Case 3

There was no clinical history available for this case. Two slides (no. 7507-LAV) were sent to the Laboratory of Pathology of the University of Michigan with the statement that they were "from an encapsulated, largely necrotic tumor of the parotid region."

The tumor might have been about 2 cm. in diameter, as judged by the sections submitted. The sections were stained with hematoxylin and eosin. One of them was restained with Weigert's iron hematoxylin.

One of the sections showed a lobulated tumor rimmed by a fibrous capsule. Outside the capsule there were some salivary gland acini and ducts surrounded by proliferating fibroblastic tissue and inflammatory cells. The remainder of the section showed almost complete necrosis except in the interlobar septa which were infiltrated by leukocytes. In the other section the tumor was encapsulated. It showed a compact structure with areas of necrosis. There were some thin-walled blood vessels, more numerous in the center of the section. The cells of the tumor were spherical, oval, or polygonal; they formed a solid mass with some suggestion of arrangement in cords and acini (Fig. 15). The cells rested on a basement membrane and stained intensely with eosin. In general, cellular detail was not very clear, but there were some areas in which the cells showed the characteristic appearance described for case 1. Their nuclei were dark, small, round, or oval, with fine granular chromatin and a single nucleolus. As in the 2 previous cases, there were none of the components usually found in "mixed tumors," and no evidence of mitotic division.

Case 4

The patient was a female, 60 years old, who had an abscess in the left parotid region in 1927. She was told that it was due to infected tonsils, and tonsillectomy and drainage of the abscess were done. In 1943 she noted a small tumor at the angle of the left jaw. This tumor grew slowly and on one occasion it was excised and drained, but it never disappeared.

On physical examination there was a firm, irregular, oval-shaped mass, measuring about 4.5 by 2.5 cm., just behind and slightly below the angle of the left jaw. The tumor was excised. At operation it appeared to be fairly well encapsulated and was the size of a large walnut. The tumor was found in the inner prolongation of the parotid gland, and there was no evidence of infiltration.

The only material available from this case was a prepared slide stained with hematoxylin and eosin (University of Michigan, no. 8325-LAX). The two sections on the slide showed an encapsulated tumor with interlobular septa. Outside of the capsule there was a small amount of normal parotid gland in which two of the intercalated ducts showed glandular structures indistinguishable from sebaceous glands and similar to those observed in case 1. In both sections there was an area of dense fibrous connective tissue in which some salivary ducts, distended blood vessels, collections of lymphocytes, and a few macrophages containing blood pigment were present. This area probably represented scar tissue resulting from the previous excision and drainage. The tumor was formed by

solid masses of cells bounded by a basement membrane and arranged in cords, lobules, and alveoli. The cells were round, oval, or polygonal and had a finely granular eosinophilic cytoplasm. The nuclei were like those in the cases previously described (Fig. 16). No mitotic figures or indications of a mixed tumor were seen.

Case 5

This tumor (University of Michigan, no. 4856-LAO) was submitted on March 6, 1937, without clinical history except that it was from the parotid gland of a male. On January 20, 1948, it was learned that the patient was without evidence of recurrence and that he had been 32 years old at the time of operation in 1937.

The size of the specimen was not recorded but it probably was about 1.5 cm. in diameter, judging by the dimension of the sections. Microscopic examination showed that the tumor was lobulated and encapsulated, with a small amount of normal parotid gland attached to the capsule. Septa of fibrous tissue separated the lobules. The tumor showed areas of compact structure, into some of which hemorrhage had occurred. In the solid areas the tumor mostly formed cords of cells arranged in single or double rows. There also were areas of acinar or glandular formation (Figs. 18 and 19). In the areas of hemorrhage the cords had been broken up, giving the tumor a pseudo-papillary appearance (Fig. 19). The tumor cells varied in shape from spherical to columnar. Other cellular details were the same as described for the other cases, except that cells with two nuclei were seen more frequently (Fig. 17). Study of many sections failed to reveal a myxomatous or chondromatous component. A few mitotic figures were present.

Discussion

A definite answer to the question in respect to the significance of oxyphilic granular cells cannot be given at the present time. Each investigator has advanced his opinion. Schaffer[12] considered them as a form of degeneration of the salivary gland epithelium. Pischinger[13] interpreted them as undifferentiated cells which, by amitotic division and further development, substituted for the destroyed glandular elements. Zimmermann[14] considered them as anomalous cells because they were not present in all persons. He also expressed the opinion that they probably were an abnormal form of cellular differentiation. Hamperl[15,16,20] interpreted them as a peculiar irreversible form of atrophy of the epithelium which also, in a broad sense, could be considered as degeneration. He emphasized the fact that the cells under discussion were associated with old age and glandular atrophy. Škorpil,[10] on Hamperl's findings

and his own, considered them to be normal in old persons and to represent an irreversible transformation of the epithelial cells into a new type. However, it is still unknown why that change takes place, and what the activities of this new type of cell may be. The presence of eosinophilic granular material in the lumina of some of the ducts, resembling the granules in the cytoplasm, suggests that they probably are able to secrete, or at least to extrude, part of their cytoplasmic content. This seems to be true both in the cases in which the cells show "adenomatous" hyperplasia and in neoplasms in which they assume a glandular structure.

Another problem with which one is confronted is to decide whether the tumors formed by these cells should be considered as true neoplasms or as a form of hyperplasia. The problem is made still more difficult by the fact that the cells involved in the process are not the normal cells of the gland, but result from the transformation of normal cells into cells of an entirely new type. The best way to answer the question is to try to reconstruct the process that may take place. Normally, for unknown reasons, but probably in relation to advanced age and glandular atrophy, the cells of some ducts, acini, or both, undergo transformation into the new type, forming isolated groups, or, in other cases, giving origin to areas of "nodular hyperplasia" which may be of solid, adenomatous, or adenomatous and papillary character.[10,11,15,16] On the other hand, usually in old persons but not always so and again for unknown reasons, the transformation of the glandular epithelium does not remain localized but extends progressively to most of the elements around the original area of epithelial transformation, or it may spread from newly formed areas. At the same time, proliferation of the cells results in enlargement of the gland. This enlargement can be explained, first, by increase in size of all of the cells involved in the process, since they are larger than the ones from which they arise, and secondly, by cellular proliferation, which in many cases does not seem to be marked, since the evidence of cellular division is limited. The result is the formation of a new structure sometimes presenting a glandular appearance, but which no longer is parotid gland or has any resemblance to it. In summary, the oxyphilic granular cells form a new and abnormal structure which arises from the gland by transformation of its cells into a new type and involves an increase in size of the area affected. Thus it seems reasonable to conclude that the process represents neoplasia rather than glandular hyperplasia.

Sometimes this tumor formation may originate in various areas of "adenomatous hyperplasia" in a single parotid gland. This occurrence may take place simultaneously or at different times, which explains the multicentric origin of some of the tumors reported, such as those of

Gruenfeld and Jorstad,[26] Harris,[27] and case 1 of this communication, all of which were formed in several nodules. It also explains the "recurrence" of the tumor reported by McFarland,[9,22,23,25] as has already been pointed out by Ackerman.[6] In trying to explain the origin of the tumor, Gruenfeld and Jorstad, Harris, and Škorpil[10] have already demonstrated that the ducts take part. In their opinions, the tumor originates from them. This is true in some cases. However, it is also true that the transformation of the cells can occur first in the acini, and in such circumstances, if a tumor develops, its origin should be considered as being from the acini. There seems to be enough evidence to substantiate the belief that all epithelial elements of the gland, with the possible exception of those of some of the excretory ducts, are liable to undergo transformation into the new cells and therefore to form the tumor. Evidence that acinar cells take part in the formation of the tumor is found in the changes observed in such cells in case 1 (Figs. 8 to 10), and to a lesser degree in case 2 of this report. The participation of the acinar component in the formation of tumor seems not to have been observed before, although it could have been expected on the basis of the findings in normal glands.

The presence of sebaceous glands, observed in ducts of cases 1 and 4, will be discussed in another communication,[32] but it should be stated now that such sebaceous glands form no part of the tumor. Their occurrence was entirely incidental.

This tumor, although rare, has characteristic features by which it can be easily recognized: It is composed of peculiar oxyphilic granular cells, which some authors have compared to the cells of the liver or adrenal[9,20,26,27]; normal ducts are absent or diminished; and there are groups of lymphocytes which do not form follicles. This tumor may present an adenomatous form with acinar and tubular formations as in cases 1 and 2, or it may adopt a more solid appearance with few areas of acinar or tubular structure, as in cases 3, 4, and 5 of this report. In the more solid form, the groups of lymphocytes may be few and small.

This tumor has been described or listed under different names, such as "onkocyte tumor," "oncocytoma," "onkocitäres adenoma," and "Hürthle cell tumor (onchocytoma)." In addition, the name "pyknocytoma" has been proposed. The term most commonly used—"oncocytoma" or "onkocytoma"—was first proposed by Jaffé[33] to designate a tumor of the parotid region more widely known by the names "papillary cystadenoma lymphomatosum" or "adenolymphoma." The term "oncocytoma" obviously is based on the name "onkocyte," given by Hamperl[15] to the cells herein described. This term "onkocyte," although probably appropriate for the reasons advanced by Hamperl,[15,20] has proved to be

confusing since the Greek root ὀγκος has been used extensively to form other medical terms with the connotation "pertaining to tumor." Interpretation of "onkocyte" as "tumor cell" is entirely different from the idea of large size which Hamperl tried to convey by this word.

It is apparent that the term "oncocytoma" should not be used to name the tumor under discussion since it is not only an unfortunate term, but it was originally devised to designate a different tumor. The term "pyknocytoma" suggested by Harris[27] does not seem appropriate because it uses the word "pyknocyte" already discarded by Hamperl[15] for reasons which he gave, and because it implies that the tumor is composed of pyknotic cells, which is not correct. Therefore, it is necessary to devise new terms which will designate more accurately these cells and the tumors formed by them, and which at the same time will not create further confusion. With this idea in mind I believe that a descriptive term such as "oxyphilic granular cells" should be used to designate the cells under discussion until future studies determine their exact nature and significance. For the same reasons a similar descriptive term should be used to name the tumor formed by these cells. "Oxyphilic granular cell adenoma of the parotid gland" or "oxyphilic adenoma of the parotid gland" can be used to designate the neoplasms composed of these cells and of the type described in this communication.

Summary

One hundred parotid glands from 51 persons were investigated for oxyphilic granular cells (oncocytes). These cells were found in 9 persons who ranged from 49 to 75 years of age. In 4 individuals the cells produced nodular hyperplasia, and in the remaining 5 they were present in the ducts or acini.

In connection with the description of 5 new cases of oxyphilic granular cell adenoma of the parotid gland (oncocytoma), their origin has been traced to cells of both ducts and acini which undergo transformation into the neoplastic type. The descriptive terms "oxyphilic granular cell" and "oxyphilic granular cell adenoma" are proposed to replace the confusing designations of "oncocytes" and "oncocytoma."

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DESCRIPTION OF PLATES

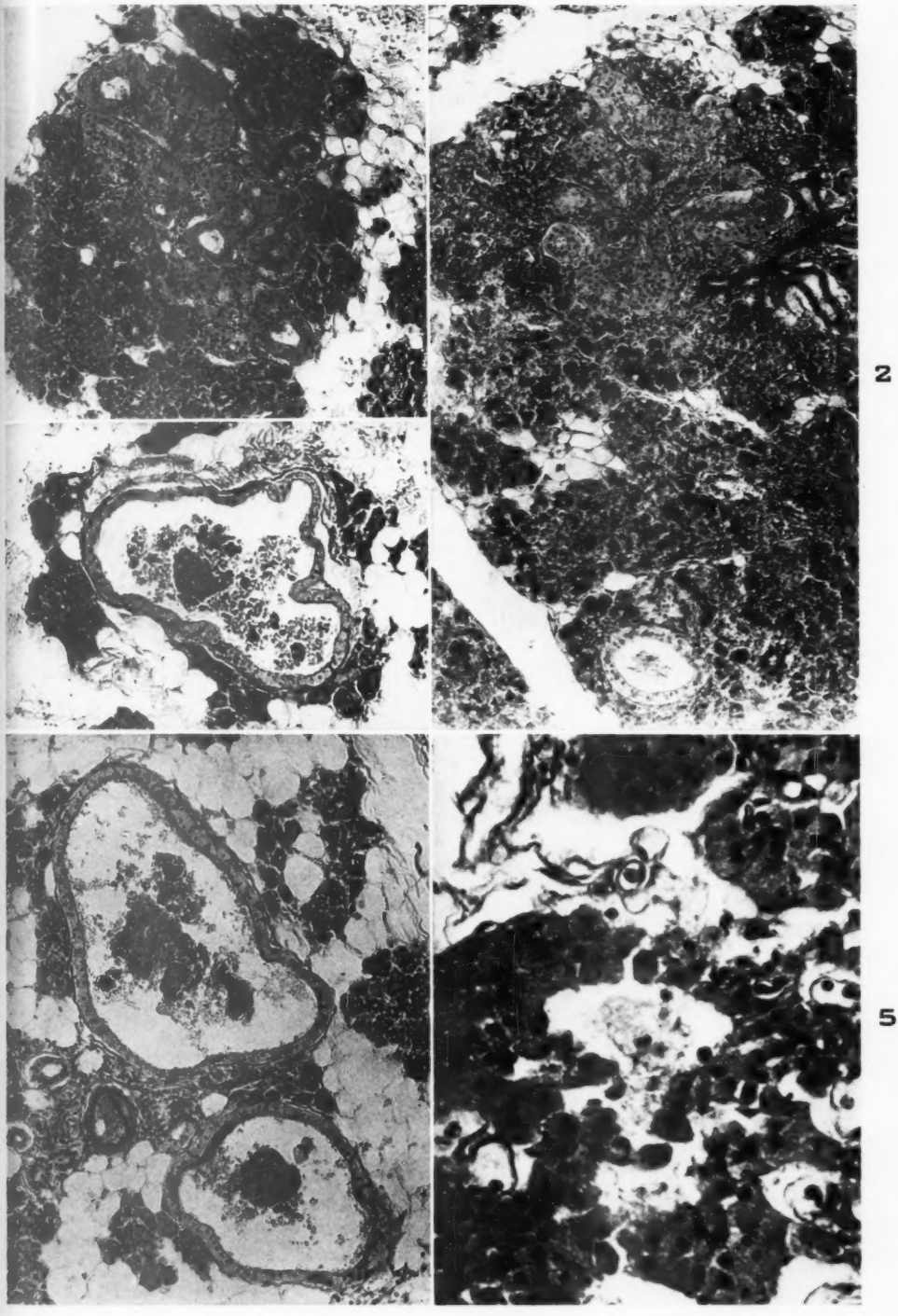
All photomicrographs were obtained from slides stained with hematoxylin and eosin, unless otherwise stated.

PLATE 76

FIGS. 1 and 2. Case 2, Table I. Oxyphilic granular cells forming "adenomatous hyperplasia" in a normal parotid gland. There is a dilated duct lined by oxyphilic granular cells in the lower portion of Figure 2. × 115.

FIGS. 3 and 4. Case 4, Table I. Normal parotid gland, showing dilated ducts lined by oxyphilic granular cells. Of note are the granular material in the lumina of the ducts and the infolding of the wall of the duct in Figure 3. × 115.

FIG. 5. Case 7, Table I. Oxyphilic granular cells from another area of hyperplasia in an otherwise normal parotid gland. × 375.



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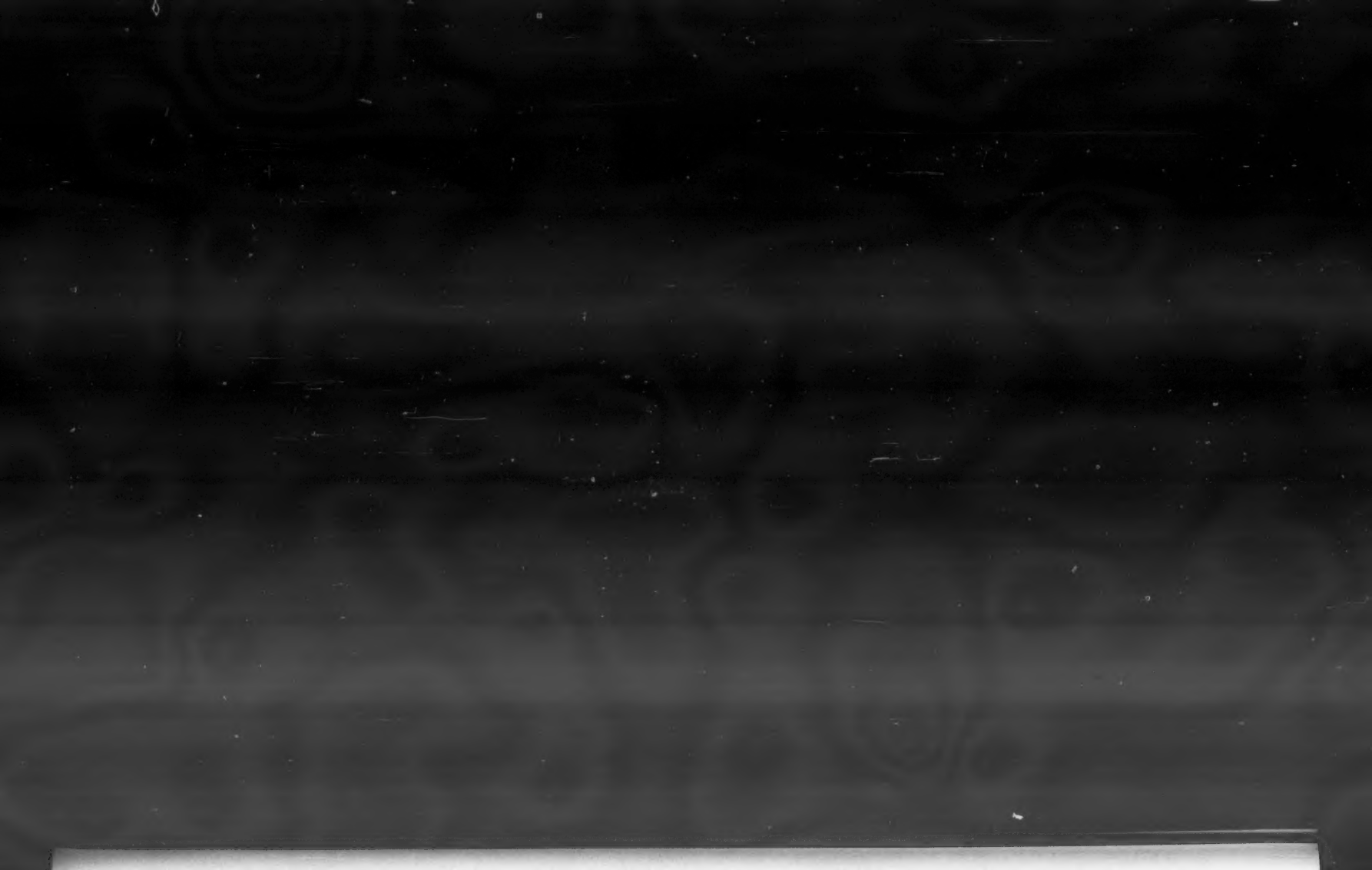
5

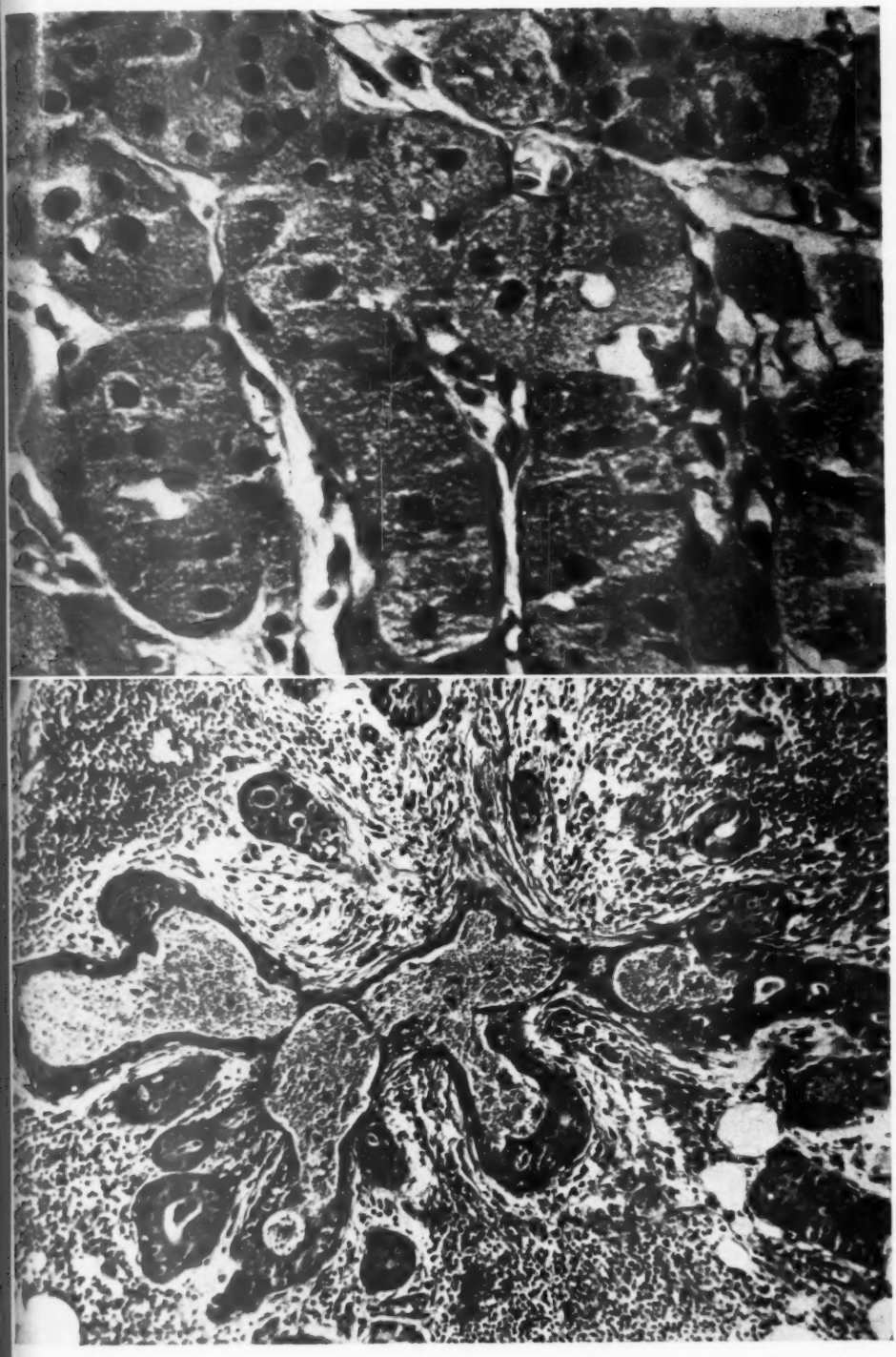
Meza-Chávez Oxyphilic Adenoma of Parotid Gland

PLATE 77

FIG. 6. Case 1. Area from the adenoma of the parotid gland in which cords of cells are seen as well as the characteristic cellular structure of the oxyphilic granular cells. Trichrome stain. × 650.

FIG. 7. Case 1. Distended duct containing oxyphilic granular material in its lumen and lined by oxyphilic granular cells in all its branches. Trichrome stain. × 100.



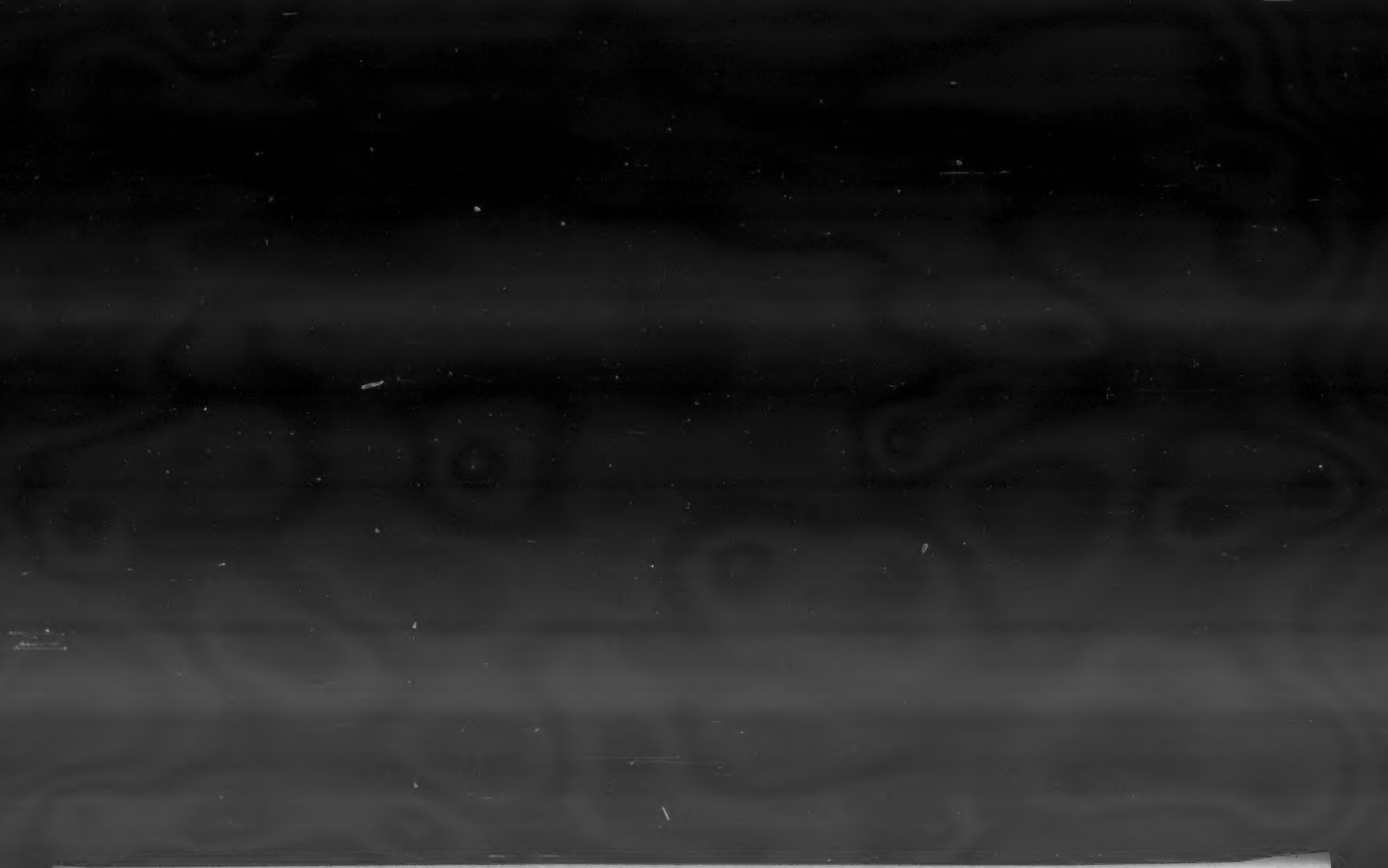


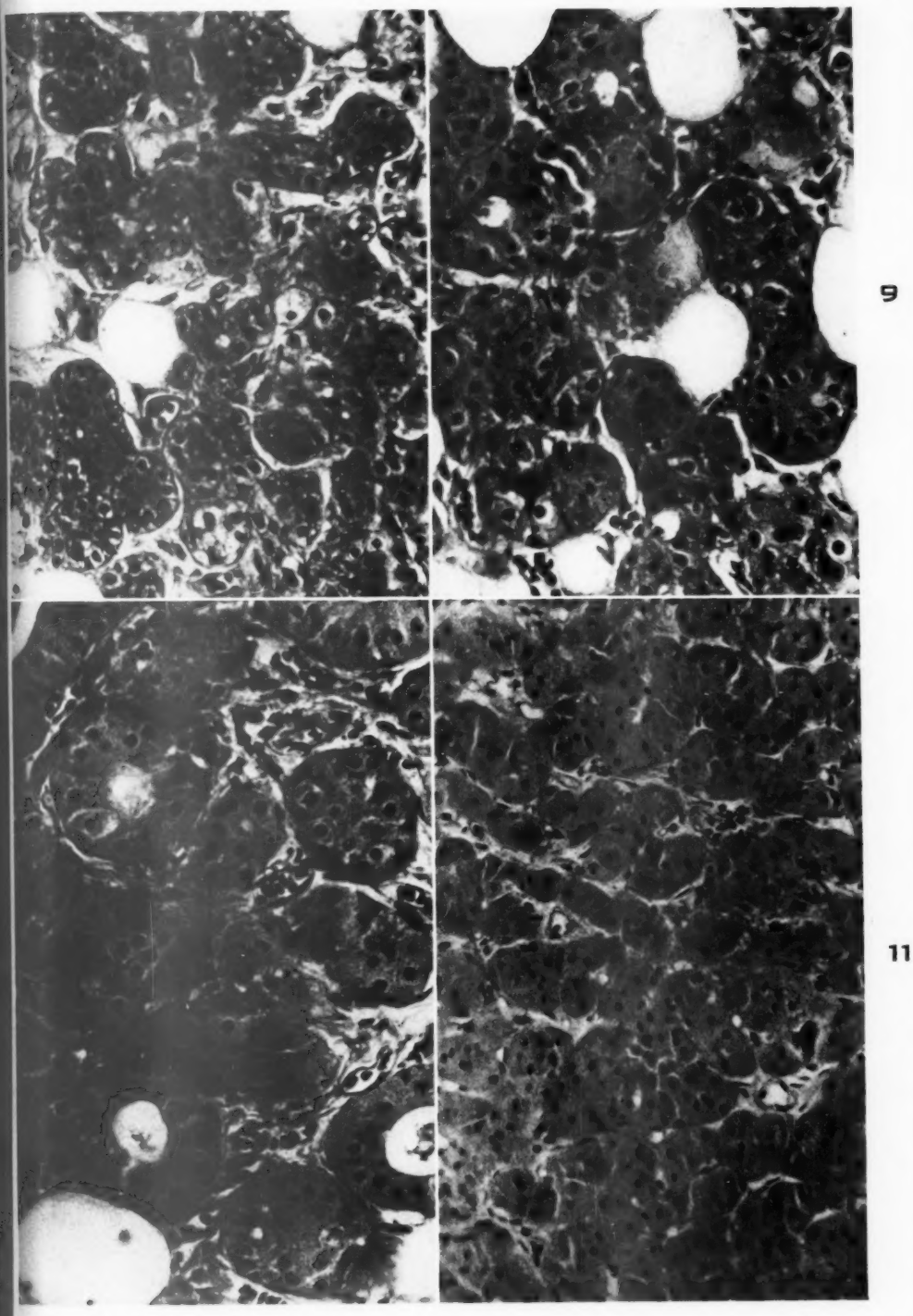
Meza-Chávez Oxyphilic Adenoma of Parotid Gland

PLATE 78

FIGS. 8 to 10. Case 1. Three stages of transformation of parotid epithelium into oxyphilic granular cells as described on page 530. × 375.

FIG. 11. Case 1. Area of the neoplasm in which the cells are forming cords composed of single and double rows of cells. × 125.





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Meza-Chávez Oxyphilic Adenoma of Parotid Gland

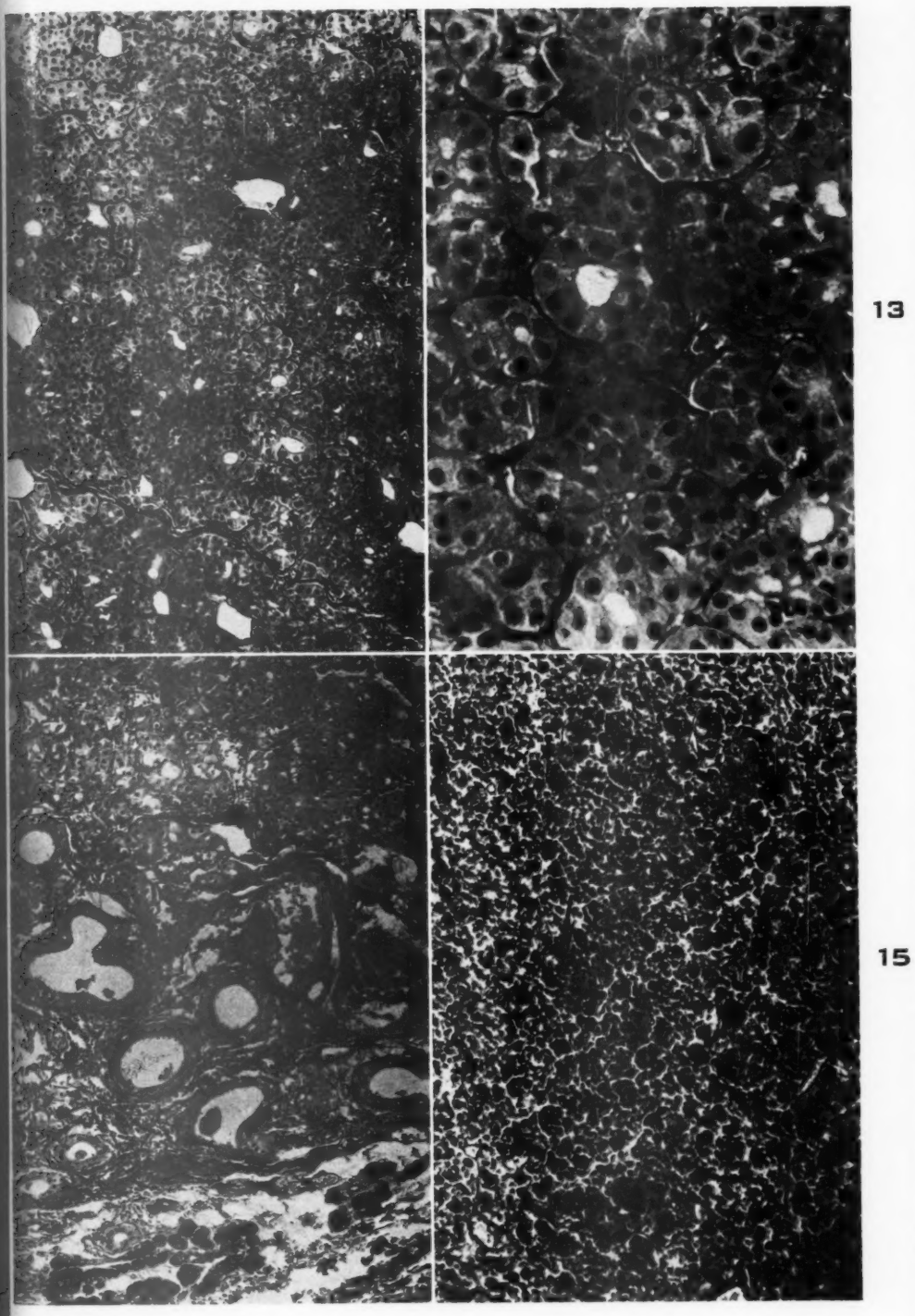
PLATE 79

FIG. 12. Case 2. Parotid adenoma. Area of the tumor showing a tubular and acinar structure. × 115.

FIG. 13. Case 2. Parotid adenoma. Tubular and acinar structure seen at higher magnification. × 375.

FIG. 14. Case 2. Parotid adenoma. Of note are the distended ducts lined by oxyphilic granular cells, the infolding of the wall of one of the ducts, and the nonneoplastic parotid gland about the periphery of the tumor. × 115.

FIG. 15. Case 3. Area from parotid adenoma. Weigert's iron hematoxylin and eosin stain. × 115.



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Meza-Chávez Oxyphilic Adenoma of Parotid Gland

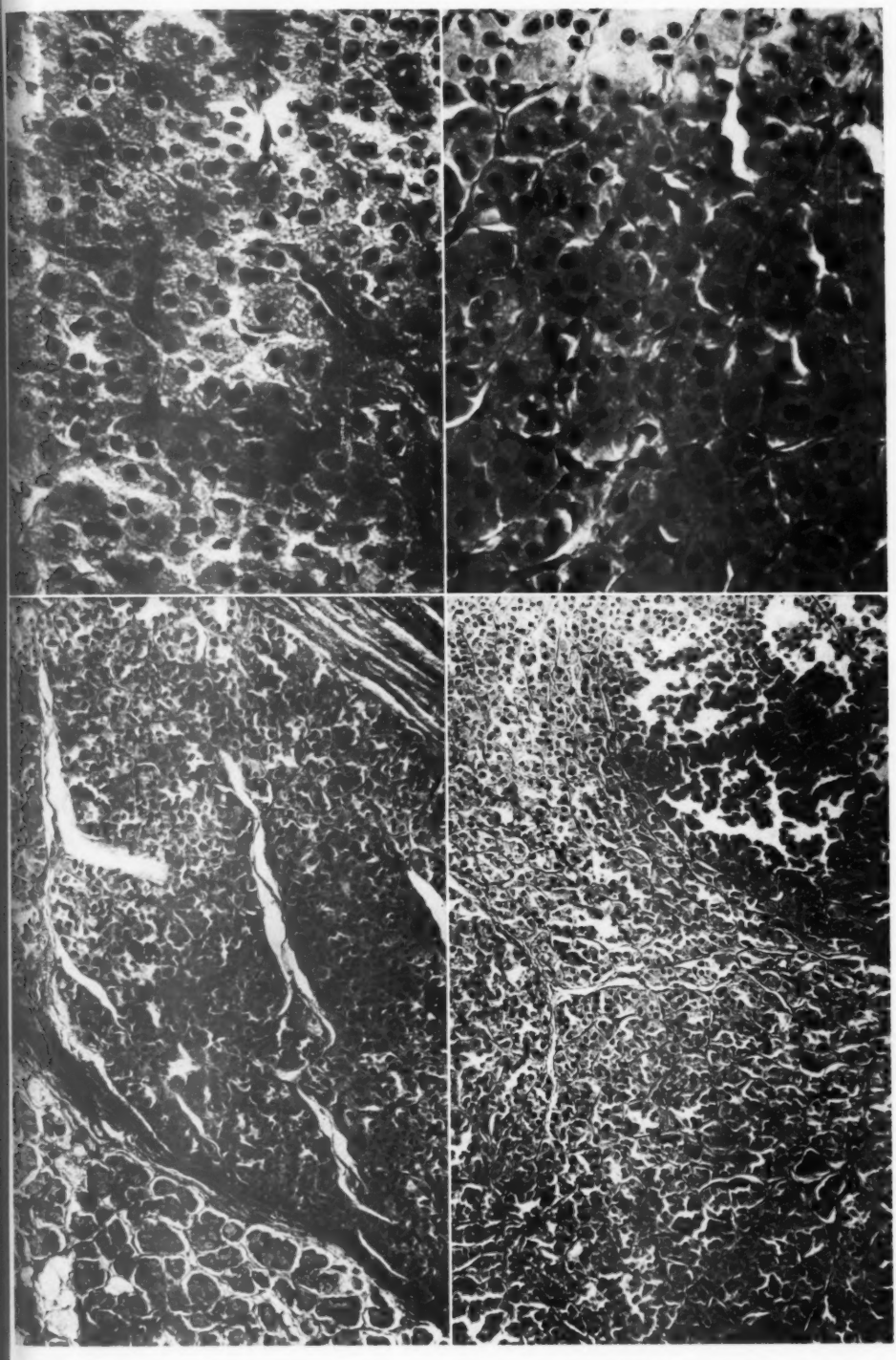
PLATE 80

FIG. 16. Case 4. Area from adenoma of the parotid gland, showing the characteristic appearance of the oxyphilic granular cells. × 375.

FIG. 17. Case 5. Parotid adenoma. In this area the tumor has a solid appearance, and there are several binucleated cells. × 375.

FIG. 18. Case 5. Normal parotid gland outside the tumor capsule. The neoplasm shows a mixed solid and acinar structure. × 115.

FIG. 19. Case 5. Portion of parotid adenoma showing a pseudo-papillary appearance. × 115.



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Meza-Chávez Oxyphilic Adenoma of Parotid Gland

INTRAMURAL FIBROMA OF THE HEART *

WILLIAM KULKA, M.D.
(From the Cuyahoga County Coroner's Office, Cleveland, Ohio)

Tumors of the heart of all types are rare.[1] As the majority of such tumors are asymptomatic, or at least without specific signs during life, they interest only the pathologist. However, some can be the cause of cardiac dysfunction or even cardiac failure and death, and thus can become of some concern to the clinician. It is assumed that the reason for the frequent lack of serious cardiac dysfunction even in the presence of extensive neoplastic involvement of the heart is due chiefly to the lack of invasion of the valves or to the slow development of the growth, giving time for compensatory changes.[2]

In the exhaustive studies and reports by Mönckeberg,[3] Mandelstamm,[4] and Yater[2] of all neoplasms of the heart which they could collect from their own cases and in a search of the literature, they mentioned only 2 which could be considered as fibroids[3] or intramural fibromas.[2] One had been described by Luschka[5] in 1855, the other by Albers[6] in 1856. Yater, however, questioned that even these 2 cases should be classified as true fibromas.

Luschka[5] found the tumor which he reported in the wall of the left cardiac ventricle of a 6-year-old boy who had died with diphtheria. He described the tumor as similar in aspect and consistency to a fibromyoma of the uterus. A definite capsule could not be discovered. The tumor measured 4.3 by 3.5 cm. Histologically, it consisted of a meshwork of interwoven columns and whorls of connective fibers and collagenous substance surrounded by, and embedded in, the cardiac musculature.

Albers[6] described tumors which he called chondroid. One of them he presented as early as 1833.[7] The second one[6] was discovered incidentally during an autopsy on a 77-year-old man whose previous history remained unknown. In this later case there was a carious process and formation of a cold abscess at the site of the 3rd to 5th thoracic vertebrae which communicated through a fistula-like channel along the ribs, with a carious process in the manubrium sterni. The left ventricle was densely adherent to the pericardium over an area of 5 by 5 cm., wherein was enclosed a hard, grayish white mass of the same size. This growth bulged 4 to 5 mm. above the surface of the heart and was separated from the endocardium by a layer of cardiac musculature 3 to 4 mm. in thickness. In the center there were only a few capillaries, but at the periphery

* Received for publication, May 20, 1948.

there was a network of vessels. In addition, Albers found in this same body a second tumor, the size of a hen's egg, which was located outside the pericardial sac adherent to the ascending aorta. On cross section, this tumor was grayish white; it was fibrotic at the periphery and showed a cheese-like necrosis in the center. From this description, it may be strongly questioned whether this last case of Albers could be classified as a fibroma of the heart.

The extreme rarity of confirmed fibromas of the heart without involvement of the valves and auricles, as evidenced by the very meager literature, prompted me to present the following case.

Report of Case

A white female infant, 8 months old and apparently in good health, died suddenly under the following circumstances: The father had played with the child and left her alone for a few minutes, returning hurriedly when he heard her cough and found her dyspneic. The baby, who had been fed about 15 minutes prior to the attack, developed hiccups and severe cyanosis. Immediate artificial respiration and administration of oxygen were unsuccessful and she died before arrival at the hospital. This sudden and unexplained death automatically became a problem for the coroner's office.

Previous History. The baby had been delivered at term by cesarean section because of cephalic-pelvic disproportion. The mother had been under progesterone treatment during the second and third months of pregnancy because of threatened abortion. The baby weighed 7 lbs. at birth and seemed well developed. She was the second child in this family; her 11-year-old brother, also delivered by cesarean section, is well developed and in good health. Other living relatives in good health include both parents, three grandparents, and one great grandfather who is 90 years of age. The death of the maternal grandfather at the age of 72 was the result of an accident.

The baby ate well and gained weight under artificial feeding, sat up freely, and had begun to stand up in the crib. She slept well, cried little, and was of a cheerful disposition. Some choking or vomiting following drinking of water or milk was considered within normal limits. Hiccups were habitual after feeding. There were occasional attacks of what the parents described as "quivering sensations" (*i.e.*, slight tremorous movements) during which both hands were stretched out and the eyes rolled in all directions. These attacks lasted only a short time and the child would be normal afterwards. The physician who attended the baby at birth and saw her four times afterwards for physical checkup and inoculation found no signs of abnormality.

AUTOPSY FINDINGS

At autopsy (no. M 2737), the body was that of a well developed white female baby, 28 inches in length and weighing 22 lbs. Ecchymoses were seen in the reddened conjunctivae. The lips were cyanotic and there was some vomited material in the oral cavity and in the larynx. Further examination revealed that the lower medial incisors had erupted recently and that the upper medial incisors were in process of erupting. The anterior fontanelle measured 1 cm. in diameter. The external aspects of the head, neck, chest, abdomen, and extremities were of normal appear-

ance. There were ecchymoses, subpleural in the thymus, and in both lungs. The lungs also showed acute congestion. The thymus measured 11 by 6 by 1 cm.

Heart. The heart (Fig. 1) weighed 60 gm. The right ventricle measured 5.5 cm. in length, 5.5 cm. wide at the base, and the thickness of the wall on cross section was 4 to 5 mm. The left ventricle measured 6 cm. in length and was 5 cm. wide at the base. Embedded in the anterior wall of the left ventricle was a hard, tumor-like mass, 5 cm. in vertical diameter and 3.5 cm. in its horizontal diameter. It extended from the anterior part of the septum through the anterior wall to the lateral margin of the left ventricle, and upward close to the annulus fibrosus of the left ventricle. The aortic and mitral valves and papillary muscles were not involved in the tumor and were of average size.

The right ventricle and right auricle were dilated. The foramen ovale and ductus arteriosus were closed. The aorta and pulmonary artery were of average size.

On cross section the hard mass was grayish white, glistening, slightly bulging, and nodular, resembling a myofibroma of the uterus. A thin layer of cardiac musculature covered its periphery. On the inside it adjoined the endocardium. The apex of the heart was not involved. No definite capsule separated the mass from the surrounding muscle tissue.

Lungs. Both lungs were acutely congested. There were a few ecchymoses in the pleura. The larynx and trachea contained some aspirated gastric material.

Spleen. The spleen was enlarged and congested.

Stomach. The stomach was filled with semi-digested material, cheesy particles, and yellowish cloudy fluid.

Liver. The liver weighed 400 gm. and was engorged with blood.

All other organs appeared somewhat congested but otherwise were of a size and appearance consistent with the age.

Microscopic Findings

Upon microscopic examination the tumor was seen to consist of interwoven columns of fibroblasts with well stained spindle-shaped nuclei and acidophilic cytoplasm. There was some collagenous stroma and, rarely, a thin-walled capillary (Figs. 2 and 3). Mitotic figures were inconspicuous. In the periphery of the mass, where the number of cells was markedly decreased, a stroma of collagenous material and connective fibers prevailed. This material extended between the overlying muscle fibers. No separating capsule-like layer could be seen. In sections stained by van Gieson's picric acid and acid fuchsin technic for demon-

stration of collagen and reticulum, a dense network of delicate fibrils was seen in the central areas; in the periphery of the tumor there were fibers of varied size and thickness with an amorphous collagenous substance interlaced with the cardiac muscle fibers. To obtain optimal photographic results, sections were stained also according to Mallory's phosphotungstic technic (Fig. 4).

Comment

These macroscopic and microscopic findings identify this tumor as a fibroma of a peculiar type. It differs decidedly from the so-called polypoid tumors of the heart and even more so from the characteristic features of myxoma.[1,2,8]

Taking into consideration the location of the tumor and its histologic characteristics, there seems to be basis for the assumption that it was derived from elements of the embryonic reticular jelly that lies between the primitive endocardium and epimyocardium.[9]

Although there were no significant attacks noted during life, it may be concluded, from the tremendous dilatation of the heart and thinning of the musculature of the left ventricle, that the tumor played a decisive rôle in the death since the reserve power of the heart must have been greatly reduced. The sudden paroxysm of coughing, vomiting, and aspiration of gastric material proved to be a greater load than this heart could sustain.

Whether the treatment of the mother with progesterone during pregnancy or the necessary roentgenologic examinations because of the cephalic-pelvic disproportion have a bearing on the formation of this congenital tumor is problematic.

Summary

Upon autopsy of an 8-months-old child, who had died suddenly, a tumor not involving the valves was found in the wall of the left cardiac ventricle, causing marked dilatation of the heart. The tumor, measuring 5.5 by 3.5 cm. in diameter, had the macroscopic and microscopic characteristics of an intramural fibroma.

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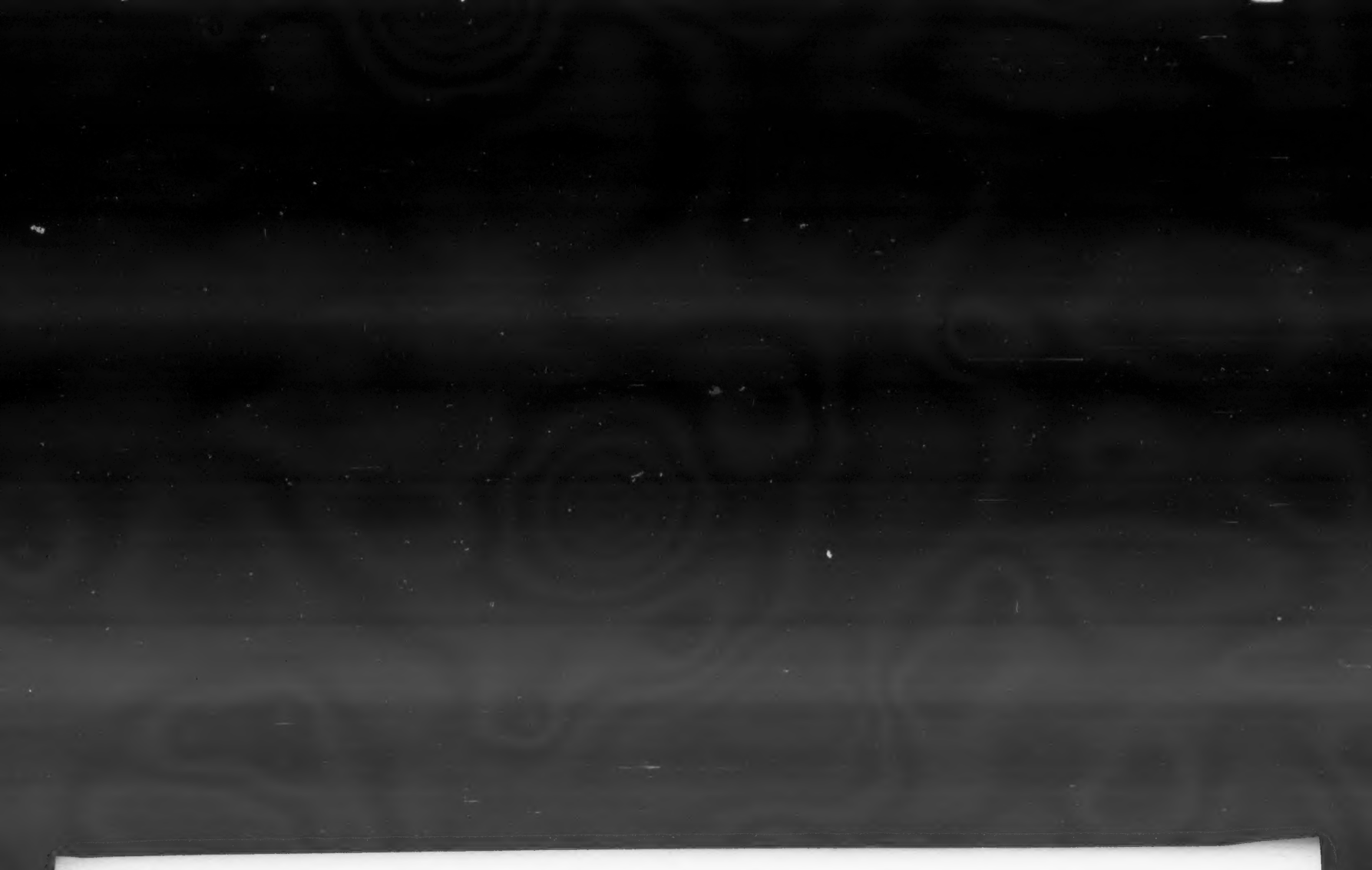
[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 81

FIG. 1. The left cardiac ventricle showing the tumor mass, the thinned layer of cardiac musculature, and the intact papillary muscles and mitral valve. The intact aortic valve is behind the tumor. × 1.

FIG. 2. Central area of the cardiac fibroma. Hematoxylin and eosin stain. × 70.



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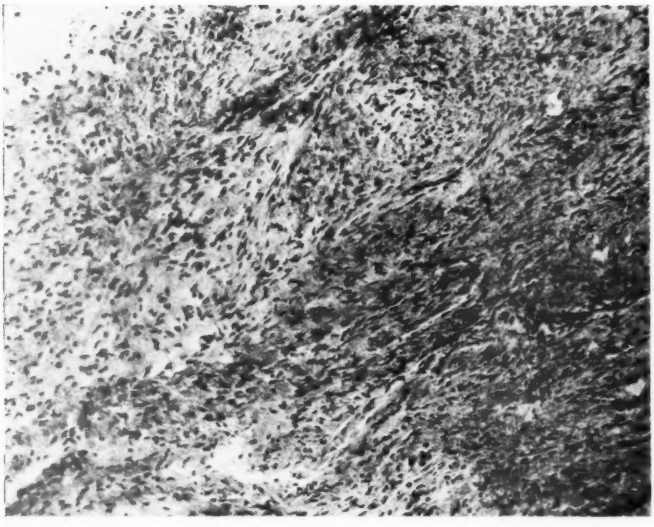

Kulka Intramural Fibroma of the Heart

PLATE 82

FIG. 3. Arrangement of fibroblasts in the center of the tumor. Hematoxylin and eosin stain. × 150.

FIG. 4. Periphery of the tumor, showing collagenous fibrils intermingled with the dark-stained cardiac muscle fibers. Phosphotungstic acid-hematoxylin stain. × 70.

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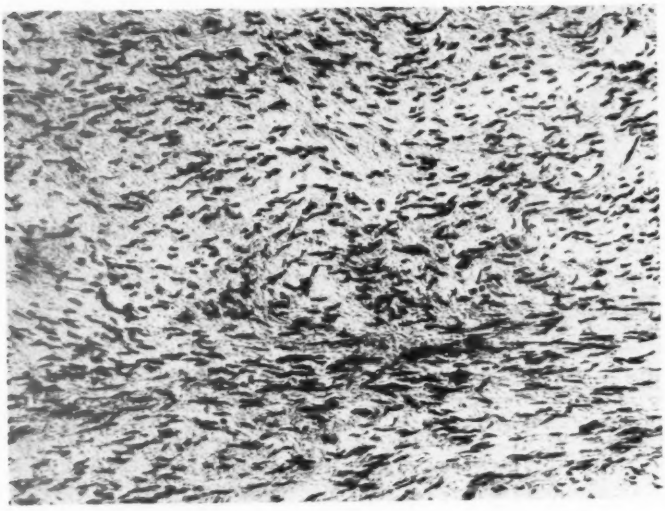

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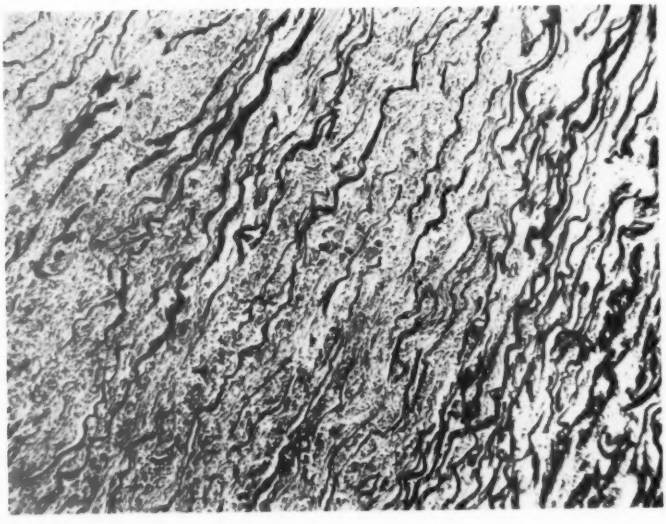

Kulka Intramural Fibroma of the Heart

MUCORMYCOSIS OF THE LARGE BOWEL *

MORRIS MOORE, Ph.D., W. A. D. ANDERSON, M.D., and H. H. EVERETT, M.D.
(From the Barnard Free Skin and Cancer Hospital, St. Louis, Mo., and the Departments of Pathology and Bacteriology, Marquette University School of Medicine and St. Joseph's Hospital, Milwaukee, Wis.)

Fungi of the family Mucoraceae, usually referred to as molds and commonly encountered as contaminants in routine culture and as saprophytes on dead or decaying vegetation, may occasionally produce infection in man. The genus Mucor has been responsible for a large number of lesions in man with perhaps *M. corymbifer* as the best known species. Gregory, Golden, and Haymaker [1] reviewed the literature and noted that in mucormycosis usually only a single organ or system is involved, most commonly the lungs or the ears. Superficial mucormycosis is not common, with only a few reported instances. Serious infection with Mucor likewise is rare although within recent years several instances have been revealed, including 4 in the central nervous system.[1,2]

The case herein reported appears to be unique in that it was characterized by mucosal inflammation and multiple ulcerative lesions of the large bowel. The lesions were infected uniformly by a fungus which appeared morphologically to be a Mucor. The involvement was extensive, actively inflammatory, and led to perforation of the bowel with death from generalized peritonitis. Although gastric ulcers infected with these fungi have been reported,[1] no previous record has been found of a widespread ulcerative colitis due to Mucor.

REPORT OF CASE

Clinical History.† The patient was a white woman, 37 years old, who was admitted to St. Joseph's Hospital, Milwaukee, complaining of generalized abdominal pain, nausea, and vomiting. Her symptoms began a week prior to admission with severe cramp-like pain in the left flank, followed by the passage of a hard mucus-covered stool. During the following days, the stools became looser, were tan, and contained large quantities of mucus. No gross blood was reported. The family physician made a diagnosis of "intestinal flu" and placed her on sulfonamide medication. The abdominal pain became generalized at the end of a week and was accompanied by nausea and vomiting.

The patient gave a history of having suffered from recurring attacks of transient gastro-intestinal disturbances during the previous 5 years. They occurred several times each year and were accompanied by the passage of mucus-covered stools. A physician had not been consulted. From her own experience she avoided greasy and fatty foods because of the distress that they caused. There was no clinical or laboratory evidence of diabetes mellitus.

On admission the patient was acutely ill. Her abdomen was noticeably distended

* Received for publication, June 4, 1948.

† We are indebted to Drs. E. G. Collins and W. Casper for the clinical information on this case.

and tender. Bowel sounds were absent. The white blood cell count was 13,300, with 21 per cent band cells, 56 per cent segmented neutrophils, 14 per cent lymphocytes, and 9 per cent monocytes. The urine contained occasional leukocytes, 1 or 2 red blood cells per high-power field, and a few crystals. A roentgenogram of the abdomen revealed a ladder-like arrangement of the loops of the small bowel and suggested intestinal obstruction or ileus. The impression after physical examination was that an acute, spreading peritonitis was present, due to a ruptured appendix or diverticulum. Surgical procedures were not attempted and a conservative course of treatment was instituted, consisting of chemotherapy, intravenous fluids, blood transfusions, and Wangensteen suction. The patient's condition rapidly became worse and she expired 8 days after admission to the hospital.

NECROPSY FINDINGS

At necropsy, 3 hours after death, the abdomen was still greatly distended but contained no palpable masses. The omentum covered the abdominal viscera and was bound firmly within the pelvis. When the omentum was released, thick, gray fluid, amounting to approximately 500 cc., welled upward into the abdominal opening. Other localized areas containing lesser amounts of purulent exudate were found throughout the abdominal cavity. A large pocket of purulent material, lying between the spleen and diaphragm, extended upward behind the pleura into the chest wall. The small bowel was dilated and its loops were bound together by recent fibrinous adhesions. The serosal surfaces of all abdominal organs were dull and appeared thickened. The appendix was not ruptured. A small rounded perforation, measuring 0.4 cm. in diameter, was discovered in the inferior surface of the cecum. The large bowel displayed numerous small, shallow, irregular, mucosal ulcers. The ulcers were present throughout the entire length of the colon and varied from approximately 0.5 to 2.0 cm. in diameter. The bases of the ulcers were pinkish white and were comparatively free from exudate. One of the ulcers had extended completely through the wall of the cecum, forming the small perforation noted above.

The spleen was soft and its capsule was thickened, especially where it formed the floor of an abscess. The pulp was soft and easily scraped from the cut surface. The remaining abdominal organs and tissues showed no significant abnormalities.

The left lung was partially collapsed and the pleural cavity contained approximately 500 cc. of clear yellow fluid. A retropleural abscess was found in the left posterior thoracic wall. It communicated with the abdomen by passing beneath the diaphragm.

Permission was not granted to examine the central nervous system.

Microscopic Examination

On microscopic examination, the epicardium was thickened and infiltrated with lymphocytes and plasma cells. A similar change was noted

in the visceral pleura, which was covered also with a thin coat of fibrin. The pulmonary alveolar spaces and the small bronchioles contained pink-staining fluid and occasional polymorphonuclear leukocytes. The splenic capsule was greatly thickened and infiltrated with plasma cells and lymphocytes. The splenic pulp was congested and contained numerous neutrophilic leukocytes. No pertinent changes were found in the liver, adrenals, kidneys, pancreas and internal genitalia.

Gastro-intestinal Tract. Multiple sections taken through the shallow ulcers of the colon presented identical microscopic appearances. The serosa was edematous, infiltrated with leukocytes, and its surface was covered with a thick, inflammatory exudate consisting of interlacing strands of fibrin and entrapped degenerating leukocytes. Lymphocytes, plasma cells, and occasional neutrophils were found scattered through the muscularis. The submucosa was three times its usual thickness, due to intense edema and an accumulation of mononuclear cells. The mucosa was edematous and in many areas necrotic. The glands and stroma in many areas were broken up into a granular mass of débris. Scattered among the necrotic tissues and in the bases of the ulcerative area were large accumulations of polymorphonuclear leukocytes and filaments of a fungus. Disruption of the muscularis mucosae and extension into the submucosa frequently were found. The same inflammatory changes encircled the fungus in the submucosa. At several points the fungus had invaded the small vessels of the submucosa and could be seen within their lumina. The vessel walls showed early necrotic changes and were infiltrated with polymorphonuclear leukocytes. In most areas extension did not occur more deeply than into the submucosa. Careful microscopic examination failed to reveal the fungus in the peritoneal exudate, in the lungs, or in other organs.

Mycologic Findings

The Mucors in general, like the Aspergilli and the Penicillia, are considered to be the weeds of mycology. They are found as saprophytes growing on dead or decaying plants or animals. Since these organisms produce numerous spores which are easily disseminated by the wind—air-borne—it is not uncommon to find them on food that we ingest or in the air that we breathe. Occasionally, these fungi may become parasitic or pathogenic as primary agents of disease in man. Usually, however, they attack man as secondary invaders, being dependent upon a preceding alteration of the tissues by infection by other organisms, chiefly bacteria, as in the case of tuberculosis; by general or constitutional disorders; by various tumefactions, notably neoplasms; or by direct trauma.

In the routine cultivation of skin lesions of various types and of sputa, it is not unusual to find these organisms as contaminants. On occasion one may feel reasonably sure that these fungi play a rôle, either major or minor, in pathogenesis. Aspergillus is the most frequent culprit, followed closely by Penicillium. Mucor is not a frequent offender but appears in a sufficient number of sputum cultures to harass the mycologist. When found producing single isolated colonies, they are usually passed over lightly. When found in abundance, however, they should be considered seriously.

It is unfortunate that cultures of the fungus responsible for the lesion described in this case were not made before the material had been placed in a fixative. The diagnosis of mucormycosis may be established with a degree of certainty on the basis of characteristics both of the tissue reaction and of the various morphologic variations of the fungus in its parasitic phase in tissue. Under these circumstances, in the absence of actively growing cultures, the organism can be identified only as to genus and not as to species. Several cases of mucormycosis, with and without the cultivation of the fungus on artificial media, have appeared in the literature. The authors agree on the general morphologic properties of the fungus in tissues, which are those of a species of Mucor; namely, that the organism has a comparatively large diameter, varying in different tissues; branching of the filaments, either abundant or not; lack of septa—coenocytic hyphae; and large accumulations of filaments in the affected tissue.

The appearance and location of the fungus in the tissue are closely linked to the state and degree of tissue reaction. The general picture of mucormycosis as observed in the sections of this case was that of an intense inflammatory process with massive areas of edema and necrosis (Fig. 1). At the periphery of the inflammatory zone was a sharp demarcation between normal and affected mucosa. Here the fungi were not seen until the area was reached where the mucosa began to show a reaction. The fungous filaments were few, appeared isolated, and were enlarged, irregular, or sclerotic. In the distinctly inflamed and hyperplastic zone the fungi were of a smaller diameter and seemed to penetrate the tissue toward the unaffected region. The filaments appeared to be very active, showing numerous branching forms which had little regard for barriers since they had invaded the lumina of the glands and had branched freely (Fig. 2). The same held true for the blood vessels, with the filaments having branched within the lumina (Fig. 4).

In the necrotic areas the filaments were abundant either as isolated large forms or as freely branching growths (Fig. 5). The hyphae ap-

peared uniform in size with actively growing, lightly staining tips indicative of young protoplasm. Cross-wall formation or septa were not seen. The presence of lightly stained areas in the filaments—vacuoles—adjacent to intensely stained segments gave at times the appearance of septum formation. In the sections, groups of filaments could be seen which on cross section gave the appearance of spores (Fig. 7). This peculiar picture was due in many cases to the irregular branching and kinking of the filaments. This characteristic was particularly noted in nodular growths in which the fungous filaments were intertwined and matted together with various inflammatory tissue cells (Figs. 3 and 6).

In summary, it may be said that although cultures of the fungus were not obtained, thus making absolute identification impossible, a sufficient number of characteristics were readily visible to suggest a close relationship to the Mucoraceae. The large size of the filaments, both in diameter and length, the method of free and irregular branching, and the absence of cross-wall formation all point to Mucor. To strengthen this view, one may refer to the publications of others in which it was stated that cultures were obtained, and that the organism, in addition to possessing the above properties, produced similar tissue changes with invasion of the lumina of the intestinal glands and of the blood vessels.

Summary

In a patient with acute colitis due to a fungus, there were ulcerative colonic lesions, perforation of the bowel, and death from generalized peritonitis. The morphologic features of the fungus, as found in the lesions produced by it, indicated that it was in all probability a species of Mucor.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

All photomicrographs were made from sections stained with hematoxylin and eosin.

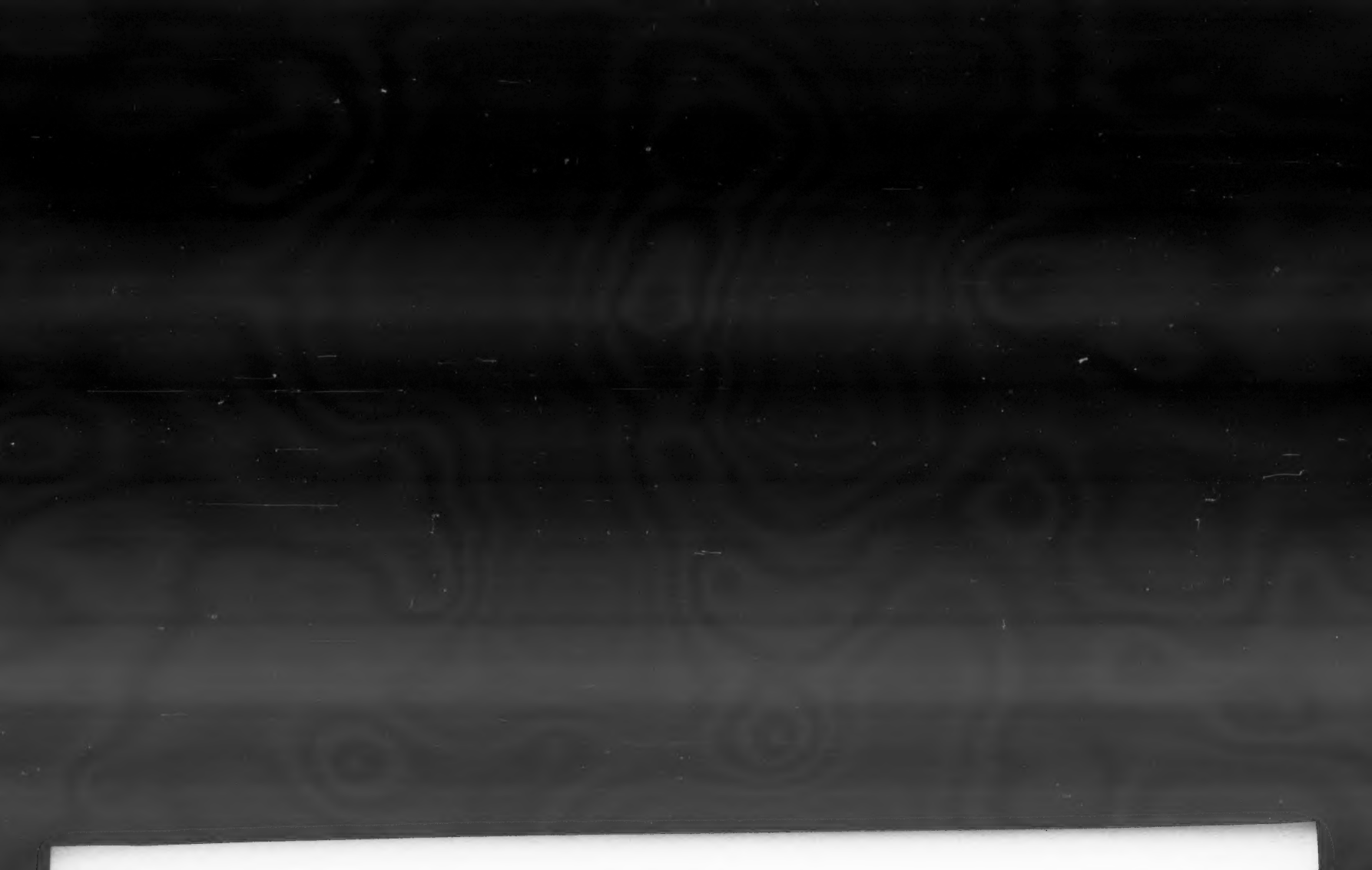
PLATE 83

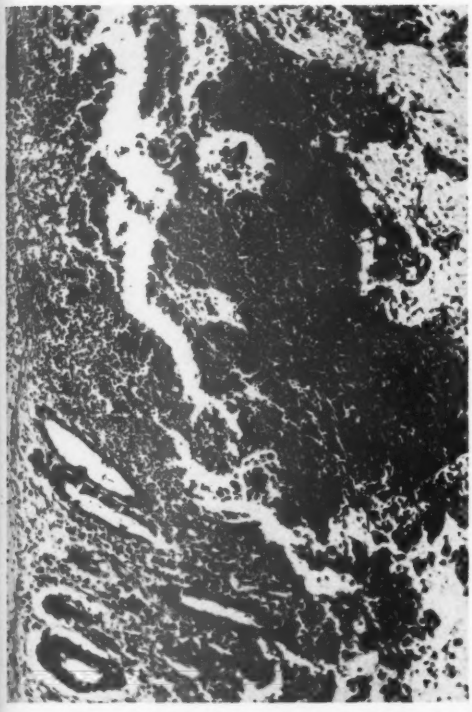
FIG. 1. Section through an inflammatory and necrotic zone of the mucosa. × 90.

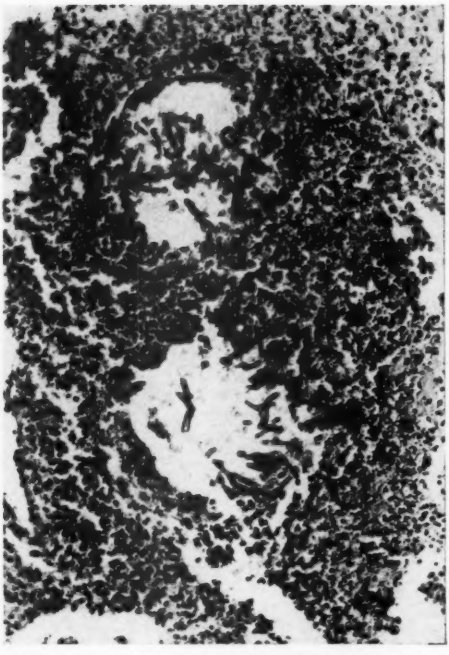
FIG. 2. An inflammatory and necrotic area, showing branching fungous filaments in the lumina of the destroyed glands. × 165.

FIG. 3. Nodule of intertwined filaments and inflammatory cells in the submucosa. × 50.

FIG. 4. Blood vessel invaded by the fungus, which has branched. × 715.







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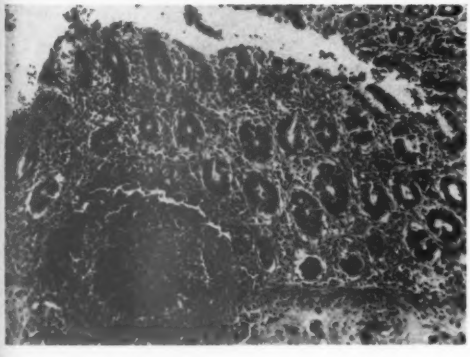

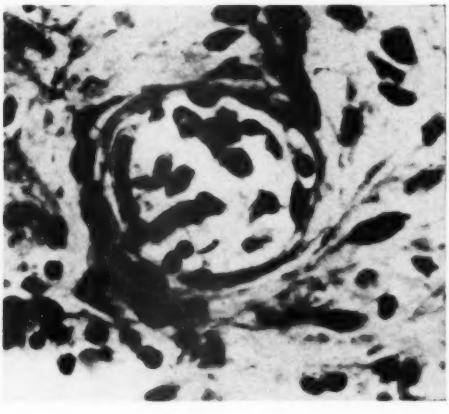

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Moore, Anderson, and Everett Mucormycosis of the Bowel

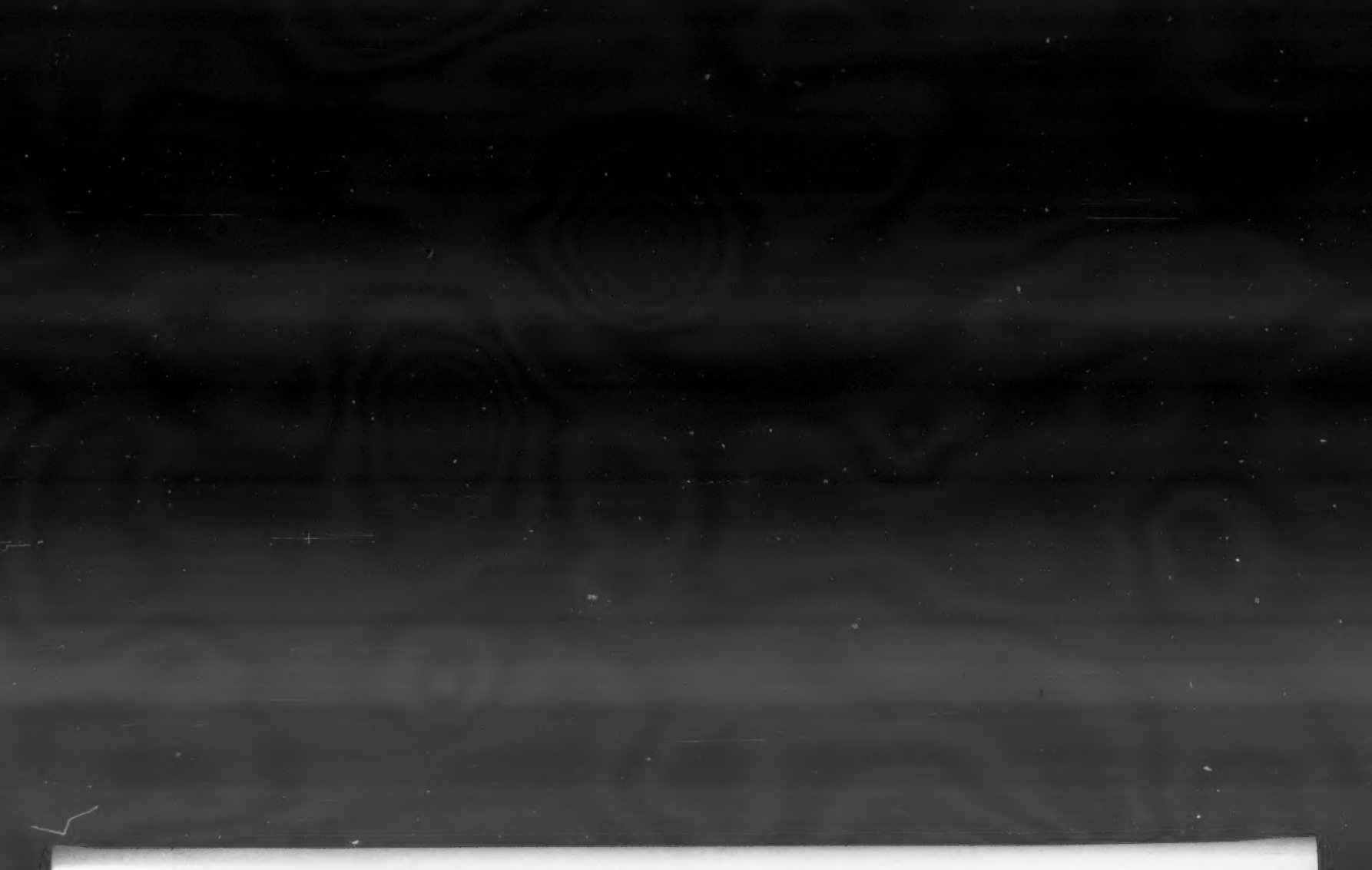
PLATE 84

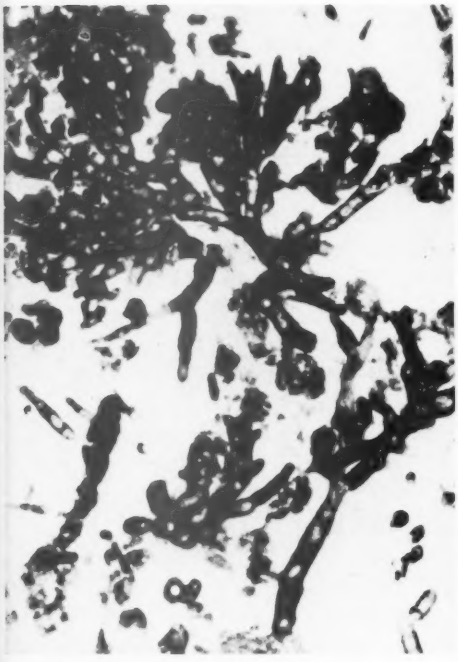
FIG. 5. Freely branching forms of the fungus in a necrotic area, showing vacuolar structure and young, growing tips of filaments. × 515.

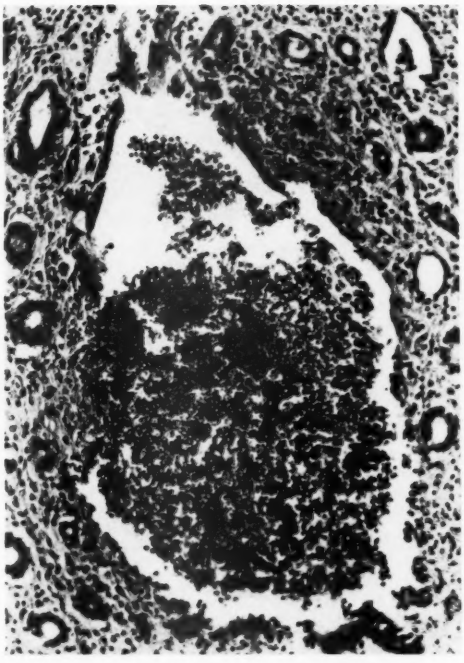
FIG. 6. Nodule of an intertwined mass of fungous filaments and inflammatory cells. Many of the hyphae appear in cross-sectional form. × 120.

FIG. 7. Fungous filaments seen in cross section. Their resemblance to spores may be noted. × 545.

FIG. 8. Young filament invading the tissue. Intense tissue response is absent. × 685.







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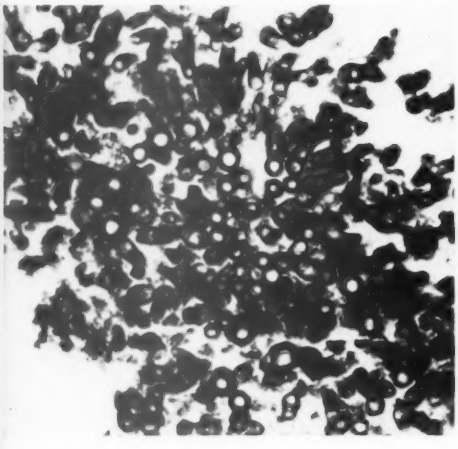

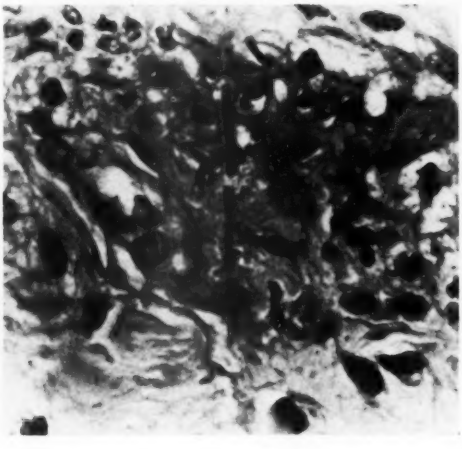

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Moore, Anderson, and Everett Mucormycosis of the Bowel

CANINE TOXOPLASMOSIS *

R. F. Langham, D.V.M., and L. B. Sholl, D.V.M.
(*From the Animal Pathology Section, Michigan Agricultural Experiment Station, East Lansing, Mich.*)

Toxoplasmosis in the dog appears to be extremely rare. Mello[1] in Italy, in 1910, described the first case. The symptoms observed in this animal were anorexia, weakness, anemia, emaciation, dry and firm skin, poor development of the skeleton, atrophy of the muscles, respiratory disturbances, diarrhea, vomiting, and a weak pulse.

Carini,[2] in 1911, upon microscopic examination of the lungs, spleen, liver, kidneys, and bone marrow of a dog, was able to demonstrate organisms having the characteristics of *Toxoplasma cuniculi*. He was able to reproduce the condition in pigeons.

Yakimoff and Kohl-Yakimoff,[3] in 1911, reported a case in a dog. The injection of an emulsion of bone marrow into 8 white mice, 4 rats, 4 rabbits, 3 dogs, and 2 pigeons produced death in the mice, pigeons, and 2 dogs, and organisms were isolated from the organs.

Nicolau and Kopciowska,[4] in 1935, found the organisms of toxoplasmosis in the brain, bone marrow, spleen, lymph nodes, lungs, small intestine, colon, and cardiac muscle of a dog dead from this disease.

Machattie,[5] in 1939, observed 2 cases of toxoplasmosis in dogs. At autopsy one animal showed marked congestion of the lungs and enlargement of the spleen, while the other exhibited necrosis of the lungs and liver.

Olafson and Monlux,[6] in 1942, reported the first cases in the dog in the United States. In addition to describing the disease in 4 dogs, they also described it in a cat and a sheep. The infection was characterized by a sudden onset, high temperature, extreme depression, and a short course. The tissues most commonly attacked were lymph nodes, liver, lungs, and intestines. The organisms occurred in a variety of cells: Monocytes, hepatic cells, vascular endothelium, smooth muscle cells, and pancreatic epithelium.

Perrin,[7] in 1943, made a study of the protozoan organisms toxoplasma and encephalitozoon, which are easily confused with each other. He injected these organisms into mice, guinea-pigs, hamsters, and rats. He used special stains for bringing out differential characteristics of the two in sections of tissue and smears.

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Hagan,[8] in 1943, gave a good summary of the disease as it occurs in animals.

The illustrative case which we wish to report is not recent, going back to 1924.

Report of Case

A young fox terrier, weighing 10 lbs., was brought to the clinic for examination. Pneumonia was diagnosed and the animal died despite treatment.

AUTOPSY FINDINGS

At autopsy the lungs were dark red and showed numerous light gray foci. These pneumonic nodules suggested tuberculosis, but no tubercle bacilli were demonstrated. The liver displayed numerous dull-gray, pin-point lesions which, with the congestion, gave it a nutmeg appearance. The mucosa of the stomach was very congested and revealed numerous ulcers, measuring from 1 to 10 mm. in diameter. Several small ulcers were present in the intestinal mucosa. There were several hemorrhages, the largest measuring 12 mm. in diameter. Other organs and tissues were somewhat congested.

Histopathologic Findings

Microscopically, the lungs showed a very acute and severe pneumonia. There was extensive congestion, and a large number of alveolar lumina contained erythrocytes. In other areas there was serofibrinous exudate. The accumulation of fibrin in the alveolar walls and lumina was somewhat patchy. Some polymorphonuclear leukocytes and macrophages were present, with an occasional macrophage revealing cytoplasm filled with toxoplasma. Several giant cells were present. The bronchi and bronchioles showed some desquamation of the epithelium and hemorrhagic exudate in the lumina.

In the liver there were very extensive areas of patchy necrosis (Fig. 1). There were numerous hepatic cells at the edges of the necrotic areas that appeared to be invaded by individual toxoplasma. A few Kupffer cells had large numbers of parasites in the cytoplasm (Fig. 2).

The intestines showed an ulcer extending into the submucosa and surrounded by an inflammatory process. With the necrotic débris were large numbers of polymorphonuclear cells, some lymphocytes, fibrin, and some hemorrhage. Outside of this zone was another characterized by large macrophages, lymphocytes, and some congestion. This inflammatory zone extended through the submucosa, muscle layers, and serosa. The smooth muscle layers showed marked atrophy and necrosis. There were numerous toxoplasma present in smooth muscle cells, and in the

cytoplasm of many macrophages and occasional giant cells (Fig. 3). The walls of some arteries, especially the media, contained them.

The organisms appeared round or oval, and were about 2 to 4 μ in length and 1.5 to 2.5 μ in width. Each had an eccentrically placed nucleus, staining deep blue. The cytoplasm was stained uniformly pink or pale blue (hematoxylin and eosin). The toxoplasma were found free, loosely grouped within the cell cytoplasm, or in compact, cyst-like accumulations. It appeared that these cyst-like accumulations were contained usually in the cytoplasm of macrophages or giant cells (Fig. 4). The isolated organisms showed their outlines a little more clearly than did those in the cysts.

After comparing the pathologic findings and the staining characteristics of the parasites of this case with those described by Perrin,[7] we consider the organism to be toxoplasma.

Summary

An acutely fatal disease in a dog was characterized by pneumonitis, gastric and intestinal ulcers, and foci of hepatic necrosis. The parasites found in the microscopic sections had the structure and distribution described for *Toxoplasma cunicula.*

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[*Illustrations follow*]

DESCRIPTION OF PLATE

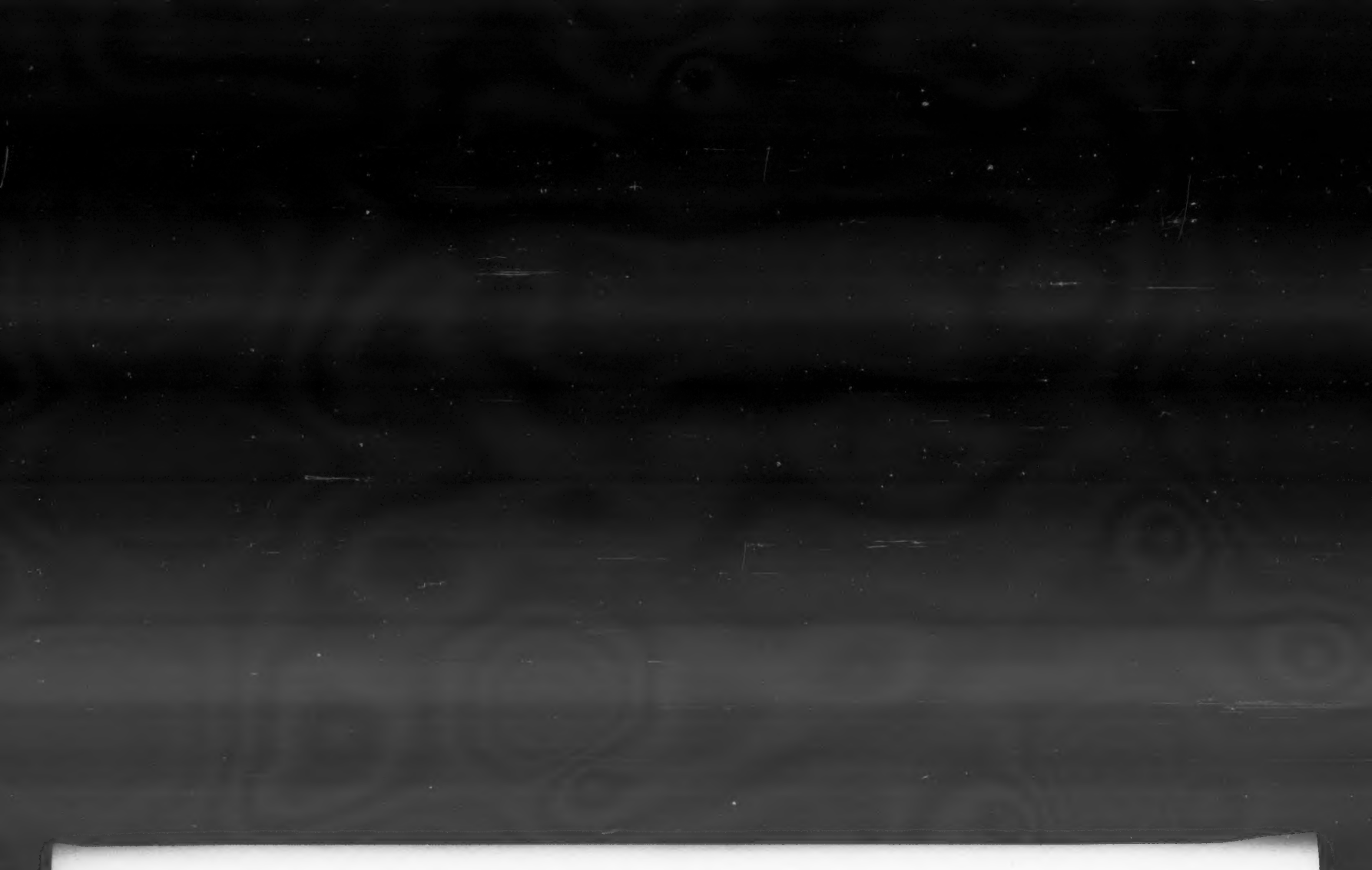
PLATE 85

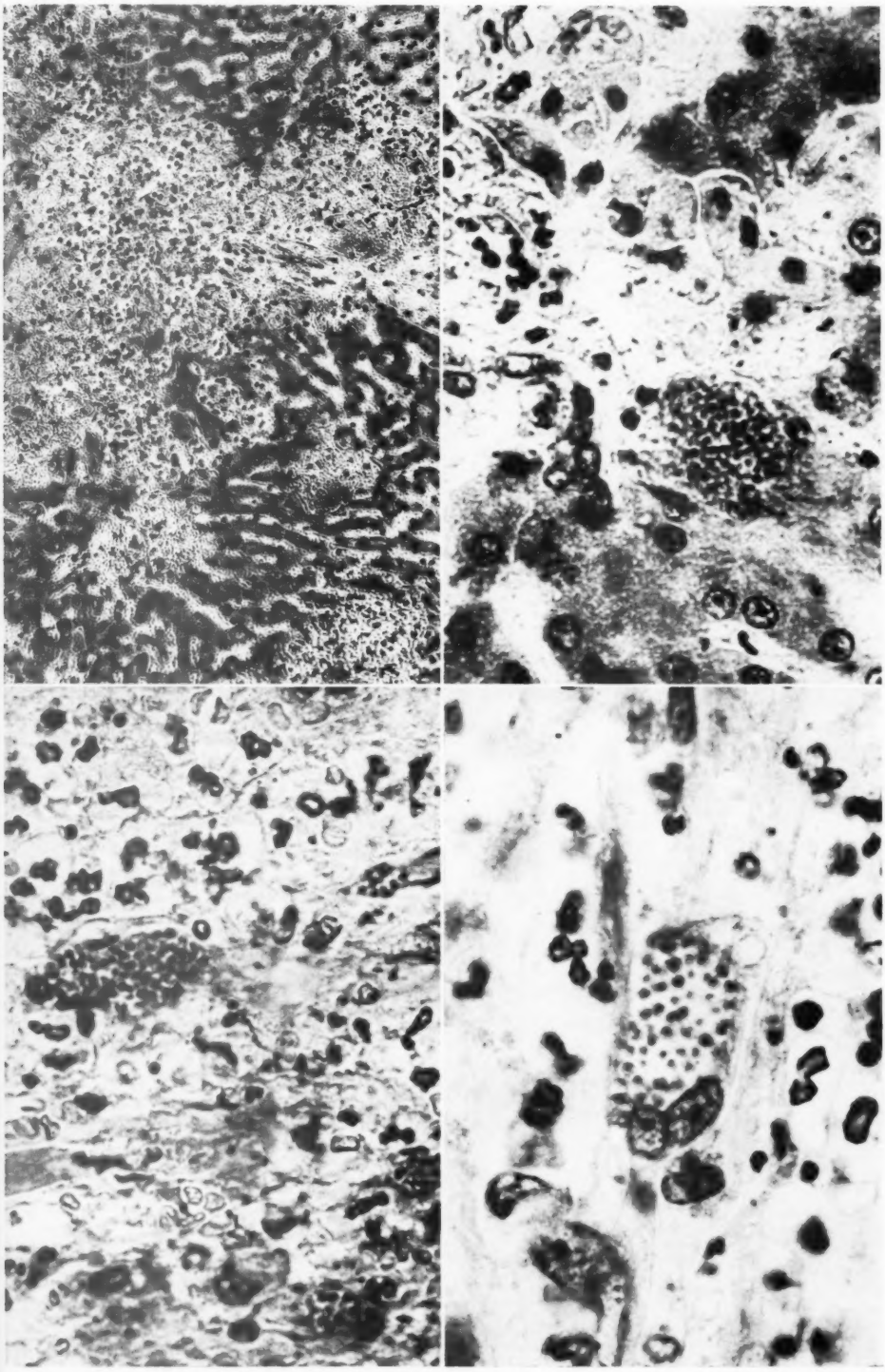
FIG. 1. Liver, showing patchy areas of necrosis. Hematoxylin and eosin stain. × 160.

FIG. 2. In the liver, a macrophage at the margin of a necrotic area is filled with toxoplasma. Hematoxylin and eosin stain. × 780.

FIG. 3. The intestinal wall shows necrosis of the muscle and macrophages containing the organisms. Hematoxylin and eosin stain. × 780.

FIG. 4. A giant cell filled with toxoplasma, as seen in the intestinal muscle. Hematoxylin and eosin stain. × 1240.





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Langham and Sholl Canine Toxoplasmosis